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PULMONARY MYCOSES AND THE DIAGNOSTIC LABORATORY*

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For many years fungus infections of the lungs have been considered rare and exotic. Because of this belief, mycotic infections have rarely been considered in the differential diagnosis of pulmonary diseases. Fortunately this situation is undergoing a definite change, motivated in great measure by such studies as those of Rixford, Dickson and Smith on coccidioidomycosis, Christie and Palmer on histoplasmosis, and by teams of investigators in the United States Public Health Service.

Many of the mycotic infections so simulate tuberculosis clinically and pathologically that it is not possible to consider one infection without the other, in making a diagnosis. It is true that some of the fungi responsible for pulmonary infections are limited to certain areas of the country, but because civilians as well as Army personnel, during the last World War, were constantly moving in and out of these endemic areas, physicians must consider the mycoses in a differential diagnosis of a pulmonary disease, regardless of the patient's present residence.

With the growing interest in mycological infections, a serious responsibility has been designated to the diagnostic laboratory: the ability to isolate and identify these pathogenic fungi. This new responsibility has created a serious problem, for many bacteriology laboratories do not have personnel with the necessary training or experience required to handle mycological cultures. Indeed, I have on two occasions been told by competent bacteriological technicians that if a fungus grows on their media, they automatically discard it as a contaminant. Such an act as

* Registry Award Paper, 1951, read before ASMT Convention, Swampscott, Mass.

this reduces the chance of diagnosing a fungus infection—not to mention the over-all efficiency of the laboratory.

On the other hand, if the laboratory technician does note the fungus growth, the question then facing her is whether or not the organism merits further study or if it can justifiably be considered a contaminant.

It is the purpose of this paper to discuss from the medical technologist's point of view the fungi most commonly the cause of pulmonary mycoses, and also those fungi which are frequently recovered from the respiratory tract and which may be considered as normal flora in most instances. No attempt will be made to go into detail about the clinical picture produced by these organisms, nor will the pathology or treatment be discussed. The methods of handling the material for culture, and the interpretation of the laboratory findings are the points to be emphasized.

Included among those fungi giving rise to pulmonary infections are *Actinomyces bovis, Nocardia asteroides, Cryptococcocus neoformans, Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Candida albicans,* and *Aspergillus fumigatus.*

ACTINOMYCOSIS: The causative agent for this infection is *Actinomyces bovis* (syn.: *A. israeli*), a branching filamentous organism, non-acid fast, and possessing a limited tolerance for oxygen but not requiring strict anaerobiosis. Actinomycosis is the most common of the severe systemic mycoses and is world wide in distribution. The etiological agent has been demonstrated in the tonsils and around the carious teeth of normal individuals, thus indicating that the infection is endogenous. *A. bovis* has not been found in nature.

Smith[1] stated that most pulmonary forms of this infection are bilateral and involve the base of the lungs, although it may occur as a unilateral lesion anywhere within the lung.

To establish the diagnosis of actinomycosis, it is necessary to find masses of gram positive branching filaments in the sputum. Aggregates of these filaments often give rise to small yellowish granules, referred to as "sulfur granules." Careful gross examination of fresh sputum may reveal these granules. A granule can be washed carefully in sterile water or saline and then inoculated into agar "shake" tubes.* Rosebury and his coworkers[2] have found Bacto brain heart infusion medium containing 2% agar more satisfactory than standard 1% dextrose infusion agar, pH 7.4-7.6. Incubation of these "shake" tubes at 37° C. for four to six days will reveal small colonies in the form of a band approximately 5 mm. wide and 1cm. below the surface of the medium. Gram stain of a colony will reveal broken fila-

* Appendix.

ments or diphtheroid-like structures. Long branched filaments, as seen in the direct smears, are not seen in culture.

If there is any question of bacterial contamination of the granules, then the agar "shake" tube should not be used, for *Actinomyces bovis* may fail to grow.

Rosebury has observed repeatedly that *A. bovis* may be isolated from heavily contaminated material, such as gingival scrapings, by culturing on dextrose agar plates and incubating in the presence of CO_2, preferably under anaerobic conditions. Positive cultures have been obtained from exudates and other materials regardless of the presence or absence of granules.

Incubation at 37° C. for four to six days will produce excellent growth of *Actinomyces bovis*. Colonies on primary isolation are rough and average about 1.5 mm. in diameter, although some colonies may be as large as 3 mm. They are white and glistening with irregular surface and present a heaped up appearance. It is very difficult to remove the colony from the agar with either a loop or a needle. Gram stain preparations made from one of these rough colonies reveal compact masses, the structure of which are short hyphae or many short fragments of hyphae, giving the appearance of diphtheroids. Long branching and rebranching filaments are rarely seen when this fungus is cultured.

The presence of granules in sputum does not indicate that *A. bovis* is present. Other infections may give rise to such granules, the more important one being botryomycosis. This infection is caused by a *Micrococcus*, but clinically may resemble actinomycosis. Gram stain of a granule from a case of botryomycosis will indicate that it is composed of gram positive cocci rather than filaments.

Streptomycin and penicillin cannot be added to the medium when attempting to isolate any actinomycete, for many strains are sensitive to these antibiotics and will be inhibited by their presence.

Ajello[3] has observed that the concentration of sputum, as for the tubercle bacillus, will destroy the systemic fungi with the exception of *Nocardia asteroides*, hence this technic cannot be used to enhance the chance of isolating a fungus. Isolation of *A. bovis* from sputum does not mean that actinomycosis is always present. Since actinomycosis is an endogenous infection, it is not impossible that the material cultured was contaminated by the fungus from some source such as the mucous membranes. Much study is still required to clarify the pathogenesis of actinomycosis. However, it is the technician's duty to report her findings, and the isolation of *Actinomyces bovis* from material sent to the laboratory cannot be considered insignificant.

NOCARDIOSIS: The pathological and clinical picture of pulmonary nocardiosis is indistinguishable from that of pulmonary actinomycosis. The difference between the two infec-

tions is a mycological one. The most common etiological agent of nocardiosis is *Nocardia asteroides*. In contrast to *A. bovis*, this fungus is a strict aerobe, growing well on Sabouraud's medium* at room temperature (18° to 25° C.). The colonies usually lack aerial mycelium, are rough with an irregular surface, and are usually a yellowish to a deep orange in pigment. Gram stain of this fungus reveals gram positive delicate branching hyphae which fragment; the diphtheroid form do not predominate as they do in cultures of *A. bovis*. Many strains of *N. asteroides* are partially acid fast. If this property is to be studied it is best to culture the organism in litmus milk.

Sputum, bronchial washings or other material is cultured on Sabouraud's medium (always incubated at room temperature) and blood agar plates incubated at 37° C. Since it is impossible to determine clinically which of the two genera may be present, the material is also inoculated to Bacto dextrose agar plates which are incubated anaerobically. In this way, either *A. bovis* or *N. asteroides* will be isolated.

Nocardia asteroides is of exogenous origin. Gordon and Hagan[4] studied a number of acid fast actinomycetes isolated from soil and vegetable matter, comparing their properties and pathogenicity for animals with those strains isolated from human infections. They did not find any cultural features that would serve to differentiate the "pathogenic" types from the soil types. However, the soil types had little or no pathogenicity for guinea pigs whereas the strains isolated from human infections produced fatal infections.

Most strains of *Nocardia asteroides*, if a large dose is given intraperitoneally, will kill a guinea pig in one week. Autopsy of these infected animals reveals acute peritonitis, abscesses on peritoneal surfaces, abdominal organs and in the omentum. Gram and acid fast stains will reveal delicate branching hyphae typical of an actinomycete.

CRYPTOCOCCOSIS: Although cryptococcosis meningitis is the most common form of this infection, it is believed[5] that primary lung infections may exist without any form of meningitis or systemic dissemination. Cox and Tolhurst stated that this is not unlikely in view of "histories of long standing respiratory or pulmonary disorders of an undetermined nature which have preceded the onset of meningitis." Pulmonary cryptococcosis may give few symptoms, and, since *Cryptococcus neoformans* is rarely looked for in sputum without a history of central nervous system involvement, it is possible that the pulmonary lesions may heal and not be seen.

Although there are no diagnostic symptoms, most cases of

* Appendix.

pulmonary cryptococcosis will have low grade fever, a mild cough with little or no sputum. The lesions may appear anywhere within the lungs. Roentgenograms are dense and resemble very closely a tuberculous lesion or a neoplasm.

The lungs are often involved following a terminal dissemination from another foci, as the brain.

When this infection is suspected, more than one sputum specimen should be sent to the laboratory, for it may take several examinations to reveal the presence of the fungus. Cox and Tolhurst[5] presented a case in which 4 sputum specimens, over a period of two to three days, were examined microscopically for *Cryptococcus neoformans*. In the fourth specimen, collected a few days before death of their patient, they were able to find a single encapsulated cryptococcus. It is interesting to note that the tubercle bacillus was found in two of these four specimens. In this laboratory the organism was cultured from two out of six sputum specimens from a patient with pulmonary carcinoma.

The sputum should be inoculated on several Sabouraud's agar slants which are incubated at room temperature and at 37° C., and to blood agar plates incubated at 37° C. This material should also be injected intraperitoneally into mice.

The etiological agent, *Cryptococcus neoformans*, is a heavily encapsulated budding yeast-like organism. It is easily differentiated from other fungi having a yeast-like appearance on the basis of its large gelatinous capsule and single bud, as well as its failure to develop a moldlike growth at room temperature.

The laboratory worker must bear in mind that there are also saprophytic cryptococci that are often seen in sputum, and it is necessary to differentiate these from the pathologic species. Although none of the saprophytic species of *Cryptococcus* possess capsules, *C. neoformans* may also lack a capsule when first isolated. This mucoid capsule may not appear in culture until after ten days to ten weeks have passed, or until a series of subcultures have been made. If *C. neoformans* is incubated at 37° C., the capsule production is sometimes accelerated.[6]

On Sabouraud's medium at room temperature, the slow growing organism is at first white and wrinkled, possessing many germ tubes. As the culture becomes older, the colony develops into a light brownish mucoid colony, which is the appearance also noted at 37° C. As a means of final identification, a saline suspension of the colony must be inoculated into mice, for virulent strains can be recognized only by animal pathogenicity tests.[7] A 0.5 ml. of a heavy suspension given intraperitoneally will usually kill mice in one to three weeks.[8] When these animals are autopsied, lesions will be seen in the liver, spleen, brain, and lungs. Microscopic examination of lesions with India ink prepara-

tions (emulsify the material in one drop of saline to which one drop of India ink has been added) or with the Hotchkiss stain[9] will reveal the budding encapsulated fungus.

Benham[7] has isolated cryptococci from human skin that are indistinguishable in culture reactions from *Cryptococcus neoformans*, but which possess little pathogenicity for animals. However, strains isolated from exogenous sources in nature differed culturally and serologically from those of human origin. On the basis of her work it has been suggested that the etiological agent for cryptococcosis may be of an endogenous source, although it has been isolated from the surface of fruits.[10]

At the present time there are no skin tests or routine serological tests used by the laboratory in diagnosing cryptococcosis. Neill and his associates[11] have developed a modified Quellung reaction for the identification of *C. neoformans* and *Sporotrichum Schenckii*, using immunologically specific antibodies. In their studies the advantage of these serological preparations was that they revealed definite capsules on weakly encapsulated forms which gave negative or questionable results when examined with India ink alone. Much work remains to be done on this technic before its practical application can be fully appreciated. It would certainly be an aid in examining spinal fluids or even bronchial secretions, but more data is needed on the species specificity.

Evans[12] has, by means of agglutination tests, capsular reaction, and agglutinin absorption, studied 19 strains of *Cryptococcus neoformans* and was able to divide them into three distinct serological types. The capsule is responsible for the type specificity.

BLASTOMYCOSIS: North American blastomycosis is a relatively common mycotic disease.[1] With the exception of an occasional case reported in Canada and one case from England, the disease is limited to the United States, most of the cases occurring in the Central and South Atlantic states.

Most cases of systemic blastomycosis begin with a pulmonary infection. Smith[1] stated that in the fatal cases studied at necropsy, 95 per cent had pulmonary infection, and in more than 50 per cent of these the most extensive lesions present were those in the lungs.

The onset of pulmonary blastomycosis is insidious and may even become disseminated before it is suspected. The patient may have a low grade fever, pain in the chest and a dry, hacking cough. As the infection progresses, the sputum will become purulent, blood streaked, and increase in amount. If the disease process is not stopped, the patient rapidly goes down hill and expires. On the basis of the bloody sputum and chest films—which may show dense masses—neoplasms are sometimes suspected. Smith and his co-workers have seen three patients with

pulmonary blastomycosis in which the diagnosis of carcinoma of the bronchus was originally made.

The diagnosis of blastomycosis is made on the demonstration of the organism in the lesions. The etiological agent, *Blastomyces dermatitidis,* is a filamentous fungus in the saprophytic stage, but assumes a yeast-like form in tissues. Sputum or bronchial washings are studied directly by means of a KOH preparation* or the Hotchkiss stain for the presence of double-contoured, budding spherical yeast-like organism measuring 8 to 15 microns in diameter. The material is inoculated on Sabouraud's agar, incubated at room temperature, and on blood agar plates which are incubated at 37° C. Twenty units of penicillin and forty units of streptomycin per milliliter of medium can be used to facilitate the isolation of the fungus.

The growth of *Blastomyces dermatitidis* on blood agar is very slow, appearing in about ten days to two weeks. The colonies are wrinkled, with a "mealy" appearance, lacking aerial mycelium. Microscopic examination of a cotton blue preparation* will show the refractile, thick-walled budding yeast-like form identical to that seen in the tissue. There may be a few mycelial fragments which indicate that the fungus is attempting to go into the filamentous form.

The fungus on Sabouraud's medium forms a downy or cottony colony with a large amount of aerial mycelium, at first white, but developing a dirty grey or tannish color as it becomes older. The characteristic feature of this organism is the conidia. These spores develop anywhere along the hyphae or even at the tips of lateral branches, as small projections which gradually enlarge. The cell wall of these projections are at first thin, but slowly begins to thicken, giving the appearance of chlamydospores. These conidia are smooth-walled, thus differentiating them from *Histoplasma capsulatum,* which can resemble *Blastomyces dermatitidis* very closely when cultured on Sabouraud's agar.

If there is doubt as to the identity of the organism, it can be injected into mice. One milliliter of a 1:200 suspension of the yeast-like cells[8] given intraperitoneally into mice will prove fatal. the time of the animal's death is related to the number of cells in the inoculum, but most of the inoculated animals should die in about two to three weeks. Necropsy of the animal will reveal large caseous nodes in the mesentery, and tubercles in the spleen, liver, and lungs. Examination of materials from any of these sites will reveal the typical tissue phase of *Blastomyces dermatitidis.*

Patients with pulmonary blastomycosis may or may not have a positive skin test which is detected by use of a vaccine* of *Blastomyces*

* Appendix.

which Martin[13] believes to be more specific than "blastomycin," although it may not be as sensitive.

At the present time, complement fixation tests are not entirely satisfactory, for the antigen used is a suspension of the yeast-like cells. However, Martin has obtained a protein fraction precipitated from these cells ruptured by sonic waves, which—on preliminary tests—appears to be an excellent complement fixing antigen.

Although *B. dermatitidis* has not been found in nature, it is believed to enter the body by way of the respiratory tract. The isolation of this fungus from pathological material is indicative of blastomycosis.

HISTOPLASMOSIS: Until 1942 this disease was thought to terminate fatally in all cases; however, there is now strong evidence that in certain parts of the United States histoplasmosis is characterized by a benign and somewhat common form. It has been through the studies of such investigators as Darling,[14] De Monbreun,[15] Meleney,[16] Christie and Peterson,[17] Furcolow, High and Allen,[18] and Palmer,[19] that the present knowledge of histoplasmosis has been attained.

There are two types of histoplasmosis: a primary pulmonary form which is characterized by being a benign infection giving rise to small calcified lesions in the periphery of the lungs, and the generalized form.

In most instances of the primary type of histoplasmosis there is no record of a previous clinical infection of the lungs. These patients give positive skin tests to histoplasmin and chest films will show calcification in lung fields and hilar lymph nodes.

Palmer[19] found in a group of 3,105 student nurses with pulmonary calcifications that these calcifications were much more frequent among those who were positive to histoplasmin and negative to tuberculin than among those who were positive to tuberculin and negative to histoplasmin. Christie and Peterson[17] and Furcolow[18] and his associates have made the same observations in their studies on children and adults. It must be remembered, however, that in only a few instances has *Histoplasma capsulatum* been isolated from cases of primary pulmonary histoplasmosis, that histoplasmin may cross react with other fungi, and finally that calcifications in the lungs are not specific for histoplasmosis or tuberculosis.

Pulmonary involvement is only occasionally the primary symptoms of generalized histoplasmosis although the lungs are almost always involved in such cases. The lesions are multiple and vary in size. There may be plueral pain but hemoptysis is rare.

In most of the earlier cases histoplasmosis was not suspected

before death but because of our renewed interest in this subject the medical profession has become more conscious of the disease with the result that many of the more recent cases have been diagnosed before death by biopsy and cultures. Indeed, from 1945 to 1949 forty two cases[20] of proved histoplasmosis have been reported.

Histoplasma capsulatum is a white, cottony, filamentous fungus when grown on Sabouraud's medium. As the culture becomes older the white gradually turns to a dirty grey or light brown. Microscopically, the culture will show branching hyphae which bear on short lateral branches small (2 to 3 mu) smooth pyriform spores. As the culture grows older some of these spores increase in size (7.5 to 15 mu), develope a rough, thick wall and become the characteristic tuberculated chlamydospores. Unless these typical chlamydospores are seen, care must be taken not to confuse the culture with that of *Blastomyces dermatitidis*, for grossly the two organisms look alike. Until *H. capsulatum* develops the tuberculated chlamydospores the two fungi also resemble one another microscopically.

On blood agar at 37° C. the colonies of *H. capsulatum* are moist, creamy, dull whitish in appearance, resembling *Micrococcus aureus*. Microscopically, the colonies are seen to be small, oval budding cells identical to those found in the lesions. If these yeast-like cells are subcultured to Sabouraud's medium and incubated at room temperature they will develop hyphae and eventually the typical chlamydospores.

To make the diagnosis of histoplasmosis blood cultures, sternal bone marrow cultures and smears must be done as well as cultures of sputum, gastric and bronchial secretions.

If sputum is present, thin smears should be made on slides or coverslips and stained with Geimsa, Wright's or Hotchkiss stain. Small, oval yeast-like cells may be seen in large numbers in the macrophages. The sputum should be cultured on Sabouraud's medium and on blood agar plates, which are incubated at room temperature and at 37° C. respectively. Howell[21] in determining the best method for isolating *H. capsulatum* from pathological material obtained eighty per cent positive cultures when he used beef heart infusion agar with ten per cent horse serum, and only forty-one and three-tenths of a per cent positive cultures if he used potato dextrose agar. Using beef heart infusion agar with horse serum or blood he obtained seventy-six per cent positive cultures when incubated at room temperature and thirteen per cent positive cultures when incubated at 37° C. He advocates that blood agar plates be incubated at both temperatures to insure isolating the fungus.

Penicillin and streptomycin are added to the medium to facilitate growth of the fungus by cutting down on the contaminanting bacterial flora.

Material collected by sternal marrow puncture should be stained as well as being cultured on Sabouraud's medium, blood agar plates, and blood agar slants which are then sealed with paraffin and incubated at 37° C.

Blood cultures should be made by inoculating several milliters of citrated blood to beef heart infusion glucose broth, pH 6.5 to 7.5.[22] Geimsa or Wright stains should also be made of the peripheral blood.

Other materials as scrappings from lesions, biopsy of lymph node or lung tissue must be carefully ground in a tumor grinder and then inoculated to Sabouraud's medium, to blood agar slants and plates as is done for the sternal marrow.

Material cultured on Sabouraud's medium may require two to six weeks incubation before yielding a positive culture while blood agar cultures may become positive in three to six days. All cultures should be held six weeks before being reported negative.

For final identification of *Histoplasma capsulatum* young mice may be injected intravenously with either the filamentous or the yeast phase. Parsons[23, 24] observed that with the yeast phase he obtained a generalized infection as early as three days. Campbell and Saslaw[25] were able to produce a high percentage of fatal infections in mice (0.5 ml of saline suspension containing approximately 3.5 million organisms) when the yeast-like organisms were given intraperitoneally with five per cent hog gastric mucin. Impression smears of the liver or spleen will show the typical tissue phase of the organism in the reticulo-endothelial cells.

A skin test for histoplasmosis is done by injecting a tenth of a milliliter of a 1:1000 dilution of histoplasmin* intradermally and reading within thirty-six to forty-eight hours. This is not a diagnostic test, having the same significance as the tuberculin skin test.

A complement fixation test for histoplasmosis can be done. This test usually becomes positive during an active progressive disseminating form of the infection. It may be considered a diagnostic test. As the patient improves the complement fixation test becomes negative.

The technic for the complement fixation test as used by Furcolow[26] is similar to the Kolmer test for syphilis. The antigen used was histoplasmin, lot H- 15, which is the same as used by several groups of workers in skin test studies.[27]

Campbell and Saslaw[27] use a ground suspension of the yeast-like cells which they find gives a clearer end point than do whole cells and their suspension does not develop anticomplementary

* Eli Lilly and Company.

activity during storage at 3° to 6° C. They advocate that this test be set up with *Blastomyces dermatitidis* antigen at the same time that the histoplasmin is used for these two fungi can cross with one another.

It is too early to make any definite conclusions on the interpretation and usefulness of the complement fixation test.

Norden[28] has developed an agglutination test using sheep's erythrocytes sensitized with histoplasmin as antigen. He modified the Middlebrook-Dubos technic[29] by using 0.25 per cent red blood cell suspension instead of the recommended 0.5 per cent.

The significance of *Histoplasma capsulatum* isolated from a patient may not always be clear cut. Furcolow[30] believes that emphasis should be placed on the observation that the fungus may be present in a wide variety of clinical conditions. "Whether it may play a decisive role as the etiological agent must be carefully considered in the light of all available evidence pertaining to the disease process." He stated that most important of all is not only to think of histoplasmosis in a granulomatous or infectious disease but that every effort should be made to find the organism and thereby establish the correct diagnosis.

COCCIDIOIDOMYCOSIS: As in the case of histoplasmosis, coccidioidomycosis produces two clinical pictures. The first is that of a primary pulmonary infection characterized by being an acute but benign self-limiting respiratory disease. Of the primary cases, approximately one to two per cent may progress to the disseminated form which can involve the viscera, bones, meninges and cutaneous tissues. This form of the disease is coccidioidal granuloma.

The etiological agent is *Coccidioides immitis* which has been isolated from soil[31] and wild rodents[32] as well as cattle, sheep and dogs. Although cases have been reported from various parts of the world, in the United States the endemic areas are in Southern California, Arizona, Texas, and New Mexico.

The symptoms of primary pulmonary coccidioidomycosis frequently are those of an upper respiratory infection although at times they may be more severe and resemble a form of pneumonia. A large number of these cases, eight to fourteen days after onset of the infection, develop numerous tender nodules, resembling erythema nodosum over the ankles or even the arms, thighs and buttocks.

There are no typical roentgenologic findings. Calcification may occur both in the hilar lymph nodes and in the periphery of the lungs. They are very difficult or impossible to distinguish from a primary infection with the tubercle bacillus. Smith[1] stated that the progressive form of the disease should be suspected if the shadows in the primary form persist for more than six weeks.

The primary form of coccidioidomycosis may go directly into the disseminated form. Invasion of the bones, joints, subcutaneous tissues, skin, viscera, brain and meninges can occur. The quantity of sputum is not large. Blood often appears in sputum as a slight blood streaking or in considerable quantity. In only three of ninety-five cases reviewed by Forbus and Bestebreurtje[33] was the sputum purulent.

Colonies of *Coccidioides immitis* on Sabouraud's or blood agar are cottony and white becoming light brown with age. They often have a "moth eaten" appearance for the aerial mycelium is not uniform throughout. The thick-walled spherules which are the characteristic form of this fungus in the tissue have been obtained *in vitro* very rarely.[34] When material is cultured at 37° C. or room temperature the filamentous or saprophytic form of *Coccidioides immitis* is obtained.

The laboratory diagnosis of coccidioidomycosis must be done with care. Cultures of *C. immitis* contain many arthrospores which are easily inhaled. This organism should never be cultured in Petri dishes but in test tubes. There are thirty six cases on record[35] in which laboratory workers have acquired infections with *C. immitis.*

Sputum, pleural fluids, bronchial secretions and other material should be examined directly in ten percent potassium hydroxide for the presence of the typical spherules. These spherules are thick-walled and non-budding (20 to 80 mu in diameter) containing endospores. Immature spherules will lack the endospores and can be mistaken for the budding thick-walled yeast-like cells of *Blastomyces dermatitidis.*

These materials are then inoculated to Sabouraud's medium and incubated at room temperature. After ten days to two weeks a moist, membraneous colony appears which gradually develops white aerial mycelium. Microscopic examination reveals branching, septate hyphae which break up into thick walled, rectangular, ellipsoidal or spherical arthospores. To make these microscopic preparations the culture should first be "dampened down" by adding to it sterile saline containing one or two drops of Tween 80.*

Smith and Baker[36] have developed a medium which inhibits the growth of all organisms except *C. immitis.*

In cases of primary pulmonary coccidioidomycosis concentrating of sputum may assist in isolating the fungus. This is done by adding enough copper sulfate to the specimen to give a final concentration of 0.04% copper sulfate and incubating this mixture at 37° C. for four hours. After centrifugation inoculate the sediment on Sabouraud's medium and blood agar.

At the same time that cultures are being made, the concentrated material should be injected intrapertioneally into guinea pigs. After one month kill these guinea pigs and examine tissues

* Atlas Powder Company, Wilmington, Delaware

carefully for the typical spherules. If no spherules are found culture tissues on Sabouraud's medium.

Most strains of *Coccidioides immitis* can be identified easily from culture but if any question of identity of the organism exists a saline suspension (CAUTION), to which Tween 80 has been added, of the mycelium can be injected intratesticularly into guinea pigs or rabbits. After one week spherules can be found in this tissue. Animal inoculation for identification is rarely needed and it is emphasized that such a step should not be done unless necessary.

The coccidioidin skin test is useful in suggesting infection but it is not a diagnostic test having the same significance as the tuberculin and histoplasmin skin tests. It is performed by injecting intradermally a tenth of a milliliter of a 1:100 dilution of coccidioidin and reading at twenty four and forty eight hours for five millimeters or more of induration. The tuberculin syringes used in the coccidioidin test should not be used for any other skin testing procedures, especially the tuberculin test.[37]

Smith and his associates[38] have developed complement fixation and precipitin tests as aids in the diagnosis of coccidioidomycosis. The complement fixation test is performed as a quantitative Kolmer test, beginning with 0.25 ml of serum. They consider complete fixation of complement necessary for conclusive evidence of infection. The precipitin tests are performed by "mixing 0.2 ml undiluted serum with 0.2 ml of varying dilutions of antigen." These tests were read daily for five days "and were considered conclusive if a button formed."

These investigators noted that the precipitins were established during the first month of illness becoming negative within three months. The complement fixation test becomes positive in action progressive disseminated infection and can, therefore, be considered a diagnostic test. As the patient recovers this titer will fall.

Not all coccidioidin preparations used for skin tests are satisfactory antigens for the complement fixation tests. Hence, when ordering coccidioidin* specify whether it is to be used for the complement fixation test or for skin testing.

The isolation of *Coccidioides immitis* from pathological material is indicative of an infection and, therefore, the laboratory technician must use all methods available to isolate the fungus and to recognize it when it is present.

MONILIASIS: Moniliasis of the lungs is a very difficult diagnosis to make. There are several species of *Candida* but only one, *Candida albicans,* is accepted as being pathogenic for man. All species, however, are found under normal conditions on the skin, in the mouth and lungs as well as in the intestinal tract. Thus, the problem

* Cutter Laboratories, Berkeley 10, California.

of determining whether or not *C. albicans* is a primary or a secondary invader in pulmonary lesions is a serious one.

Negroni and Doglio[39] examined 212 sputa from patients with various infections, seven bronchial secretions, two biopsies of the mucous membranes, one trachial fluid, one pleural fluid and one biopsy of the lung for fungi. Sixty percent of their cultures were positive of which eighty three and a half percent contained species of *Candida*. Twenty six of the isolates were *C. albicans*.

Haley and McCabe[40] studied the mycological flora of seventy one autopsies of individuals in which no mycological infection was suspected. Thirty eight (53.5%) of these autopsies yielded positive cultures for fungi. *Candida albicans* was the fungus most frequently isolated and it was found in the lungs only.

In a series of sixty nine sputa and bronchial washings from patients not suspected of having a fungus infection, cultured in this laboratory, twenty five were positive for *Candida albicans*, thirteen of which were from tuberculous patients and two from cases of carcinoma of the lungs.

Bronchial moniliasis is the more common form. A cough is the characteristic symptom. The patient is not seriously ill, and the sedimentation rate is rarely elevated. The sputum is colorless, mucoid, and will frequently be found to contain "flakes" composed of budding yeast cells. Castellini reported that this was a common infection found in tea tasters of Ceylon. Roentgenograms may show only a non-specific type of peribronchial thickening and are not diagnostic.

Pulmonary moniliasis is a more severe form, but is not as common as bronchial moniliasis. Temperature and pulse are elevated and pleural pain is common. The sputum is mucoid, gelatinous, and is often blood streaked. Areas of infection are of the lobular or bronchopneumonic type and are frequently scattered in two or more lobes.[1]

To diagnose moniliasis, the budding yeast-like organism must be found repeatedly in the direct examination of sputum. The sputum must be a freshly collected specimen, preferably one collected early in the morning before the patient has had breakfast. Sputum or exudates should be inoculated on Sabouraud's medium (room temperature) and blood agar (37° C.). The method of Conant and Martin*[41] is used to identify the isolated species of *Candida*.

Lewis and Hopper[42] have shown the skin test for moniliasis to be of little value as they obtained positive reaction in 54% of patients without moniliasis of any kind. Their observations have been substantiated by other investigators.

Serological tests are of little value in making a diagnosis, for

* Appendix.

Martin, as well as others, found that there is a large degree of cross reaction between the species of *Candida*. Conant[41] stated that the presence of agglutinins, precipitins, or complement fixing antibodies may serve as proof of invasion of the body by *C. albicans*, but it is not possible to state whether the invasion is primary or secondary.

Thus, the significance of *C. albicans* in sputum or bronchial secretions must be interpreted with caution. The laboratory data must be used in conjunction with the clinical findings, for, alone, they cannot make a diagnosis of moniliasis.

MISCELLANEOUS FUNGI: *Aspergillus, Penicillium,* and *Mucor* species are often isolated from the sputum. They are frequently found in sputum of patients with chronic bronchitis and asthma. These are common molds found throughout the world and, in most instances, they must be considered as contaminants.

Vaccines made from these fungi will often give a positive skin test since the organisms can be normal flora of lungs, intestinal tract, skin, and even the ears. Thus the significance of a positive skin test is nil, with rare exception.

To diagnose a primary infection by one of these fungi before death is difficult. The symptoms are similar to that of pulmonary tuberculosis. Chest films are not characteristic, which is so often the case in pulmonary mycoses.

ASPERGILLOSIS: Aspergillosis is most frequently limited to the pulmonary system, and invasion of other organs is very infrequent. There are two cases of general aspergillosis in the literature.[43]

Repeatedly finding *Aspergilli* in the sputum does not make the diagnosis of aspergillosis, for it cannot serve as conclusive evidence that the fungus is a primary invader. *Aspergillus fumigatus* is the species most frequently associated with pulmonary infections.

Sputum is examined by means of KOH preparations for the typical conidiophores of *Aspergillus*. It is then cultured on Sabouraud's medium at room temperature. The "tissue phase" of this fungus, as well as *Penicillium* and *Mucor,* is the same as the saprophytic phase, as far as is known.

Aspergillus colonies develop rapidly; and within two to three days will appear as white cottony mycelium which quickly becomes a green to dark green—or even black—in color. Microscopic examination of the colony will reveal the typical swollen conidiophore with its sterigmata. The spore chain will be broken in the preparation of the slide, and the spores will be seen floating free. To identify a species of *Aspergillus* it is necessary to use the key devised by Thom and Raper.[44]

Since pulmonary aspergillosis is frequently a secondary infection in tuberculosis, bronchiectasis, and carcinoma of the

lungs, it is necessary to rule out these infections before one can consider primary aspergillosis.

PENICILLOSIS: Pulmonary penicillosis has been reported on several occasions,[41] but what has been said for aspergillosis also applies to this infection. The key to Raper and Thom[45] is necessary to identify a species of *Penicillium.*

MUCORMYCOSIS: Several cases of pulmonary mucormycosis have been reported.[41, 46] Murphy and Bornstein[46] stated that "it seems a general predisposition (such as diabetes) or local primary disease (as pneumonic or infarction) exists" before *Mucor* can cause an infection in the lungs. They offer this as the explanation for the rarity of infections due to the ubiquitous molds.

Mucor, like *Aspergillus* and *Penicillium,* is a common laboratory contaminant. Thus Conant[41] believes the diagnosis of mucormycosis can only be made when the evidence is overwhelming.

On Sabouraud's medium the fungus is rapidly growing, filling the tube in four to five days with abundant growth of floccose aerial mycelium that is dark gray. Microscopically one sees that the typical sporangium is the result of the swelling of the terminal end of the sporangiophore. The sporangium (sac) soon becomes filled with endospores as a result of cleavage of the protoplasm within.

APPENDIX

1. **Agar "Shake" Tube:** A tube of beef or veal infusion glucose agar, pH 7.6-7.8, is heated to liquefy the agar and then cooled to 40° C. Granules should be crushed between sterile glass slides and the material then inoculated into several of these "shake" tubes. Incubate the tubes for three to four days and then examine.

2. **Sabouraud's Glucose Agar:**

Dextrose	40 grams
Agar	35 grams
Peptone	10 grams
Distilled water	1000 ml.

Autoclave at 15 lbs. pressure for 15 minutes and slant. It is not necessary to check the pH.

3. **Corn Meal Agar:**

62.5 grams of yellow corn meal
1500 ml. of distilled water.

Heat for one hour by running in the autoclave without pressure, or at 60° C. for one hour in a water bath.
Filter through absorbent cotton.
Bring to original volume; add 13 grams of agar per 1000 ml. of broth. Tube and autoclave at 15 pounds for 8 minutes.

4. **Beef Extract Broth for Carbohydrate Studies:**

Difco beef extract	3 grams
NaCl	5 grams
Peptone, Difco	10 grams
Water, distilled	1000 ml.

Indicator: bromcresol purple (1.6% in 95% ethyl alcohol): 1 ml. per liter of medium.

5. **Stock Sugars for Fermentation Studies on Candida Species:**
Make 10% solutions of dextrose, maltose, sucrose, and lactose. Sterilize by autoclaving or filtering through a sintered glass filter. Add enough of each sugar aseptically to the above extract broth to give a 1% concentration.

6. **Lactophenol Cotton Blue:**

Phenol crystals	20 grams
Lactic acid	20 ml.
Glycerol	40 ml.
Distilled water	20 ml.

Dissolve by heating gently under a hot water tap. Add 0.05 grams of COTTON BLUE. (POIRRIER'S BLUE, National Aniline Division)

7. **Cotton Blue Preparation:** Place one drop of the lactophenol cotton blue on a slide; into this place a small portion of the fungus growth—tease gently and thoroughly. Cover with a coverslip and examine.

8. **Potassium Hydroxide Preparation:** Place one drop of 10% potassium hydroxide on a clean slide. Tease a little of the material to be examined in this drop and place a coverslip over the preparation. Heat this preparation gently for several seconds, being careful not to "boil" it.

9. **Preparation of Vaccine:** [After Conant *et al.*[4]]
Scrape the fungus growth from the surface of the agar and place in a sterile mortar. Grind the material after adding a little sterile physiologic saline. The suspension should then be diluted with saline to give the density of a light "ground glass" appearance. The vaccine is heated in a 60° C. water bath for two hours. Before use, sterility tests must be done, culturing for fungi and bacteria.

The fungus may be washed from the agar slants and heated without being ground. In such an instance it may be necessary to heat the vaccine for a longer period of time. Care must be taken, however, not to denature the vaccine.

10. **Identification of Candida Species As Suggested by Conant[4] and His Associates:**
Isolate the fungus on Sabouraud's medium. Transfer the fungus to Sabouraud's glucose acid broth and incubate at 37° C. for 48 hours. After noting the type of surface growth, shake the tube and inoculate a beef extract blood agar plate which is then incubated for 10 days at 37° C.

Note the type of growth on the blood agar plate and then subculture one of the colonies to a beef extract agar slant, pH 7.4. Subculture this growth for two or three generations. Inoculate a Petri dish containing corn meal agar by cutting the agar with the inoculating needle. Incubate this plate for several days at room temperature and then examine carefully under the microscope for the details of mycelial growth.

At the same time that the corn meal agar is inoculated, inoculate four beef extract broth tubes, containing 1% glucose, maltose, sucrose, and lactose respectively with a saline suspension of the organism from the last transplant on beef extract agar slant, seal with sterile vaseline and incubate at 37° C. Examine in 48 to 96 hours for fermentation reactions.

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BONE MARROW IN HEALTH AND DISEASE*

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The bone marrow, the largest organ in the body, varies greatly in its volume in the normal human. At birth all of the bones are filled with red marrow and are active in the process of blood formation. As an individual ages, this red marrow is gradually replaced in all of the long bones by yellow, fatty marrow and in normal adult life hematopoiesis is carried on almost entirely by the bones of the thoracic cage, skull, vertebrae and pelvis. The marrow obtained from these sources is not homogeneous in its consistency and a single bone marrow puncture can give an extremely false impression of the true make-up of the marrow as a whole if the needle happens to enter at the site of an island of cells of any one type or should penetrate an area of relative hypoplasticity. This is probably the reason for the wide variation of the published figures on normal bone marrow differential.

Smears of the bone marrow may be prepared in several ways depending, in part, on how the material was obtained. If a puncture is carried out, the liquid may be dropped directly upon slides and gently smeared, or particles of marrow may be lifted out before the blood coagulates and impressions made with these. Some workers prefer to mix the aspirate directly with heparin and then smear the bits of marrow material. When a biopsy is performed, the curettings are pressed gently against a slide and impressions made.

As soon as the material is dry, it may be stained, using Wright's method, and then counterstained with dilute Giemsa stain. The time for counterstaining varies from twenty to thirty minutes depending to a large degree on the cellularity of the specimen, a highly cellular marrow such as that seen in myelogenous leukemia taking the longest time.

The first step in the study of a bone marrow slide is to scan it carefully, using a low power objective and keeping in mind three factors: the number of megakaryocytes, the presence of any clumps of foreign cells and the general composition of the marrow. The megakaryocytes are found chiefly at the edge of the smears and, if not found on one slide, should be searched for on several, if necessary. Malignant cells are frequently seen in clumps or rosettes and usually can be readily recognized. A marrow which is composed of many cells of uniform type is immediately suspected of showing some blood disturbance.

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Ordinarily, the general bone marrow picture is apparent from a careful study of several slides, but if a differential count is made, at least 500 cells should be tabulated and several smears observed.

The normal bone marrow is composed largely of cells of the granulocyte series and the majority of these cells are in the myelocytic and metamyelocytic stages. An occasional white blood cell blast and usually 1 to 2 per cent of lymphocytes are seen. Reticuloendothelial cells and monocytes are rarely encountered in the normal marrow. Numerous nucleated red blood cells are seen scattered throughout the smears and the ratio of white blood cells to red blood cells varies greatly but is usually between 2:1 and 8:1.

Regardless of the nomenclature used, all workers agree that there is a primitive cell in the erythroid series from which the normal and the abnormal red blood cells develop. This cell may be called the erythrogone and from it stem the normoblasts, developing into the normal adult red cells, and the megaloblasts, producing the large red cells of liver deficiency anemia. The erythrogone is a large cell approximately 20 microns in diameter, with a deeply basophilic cytoplasm and a large meshy nucleus which fills most of the cell and usually contains nucleoli. These cells are found in scanty numbers in normal bone marrow but are greatly increased in marrow showing red blood cell hyperplasia, whether of a normoblastic or megaloblastic type.

The cells in the normoblastic series are classified as normoblasts A, B or C. Normoblast A is the youngest form, with a deep blue cytoplasm and a nucleus whose chromatin shows more condensation than that of the erythrogone. The nucleus of normoblast B displays a much heavier clumping of its chromatin material and the cytoplasm varies in color from a dark gray-blue to a light green-blue depending upon the amount of hemoglobin in the cell. The cell in the C stage is fully hemoglobinized and contains a small pyknotic nucleus of either purple or black coloring.

The megaloblastic series differs from the normoblastic in that at each stage of development the nucleus appears to be retarded in comparison with the cytoplasmic changes; that is, in the megaloblast B stage, the cytoplasm shows definite color changes of hemoglobinization, yet the chromatin arrangement of the nucleus is much like that of a normoblast A. The megaloblast C may show little difference from the normoblast C except for its larger size.

The bone marrow of pernicious anemia shows a marked preponderance of megaloblasts, a definite increase in the number of erythrogones and, occasionally, mitotic figures. Many of the early megaloblasts, particularly, are of larger size than would be seen in normal cells at the same stage of development. Another

feature is a relative decrease in the cells of the granulocytic series and the presence of large atypical metamyelocytes containing nuclei almost doughnut-like in shape.

Any condition in which there is a constant drain on the red blood cells, whether from increased destruction as in hemolytic anemia or from chronic blood loss, results in marked hyperplasia of the bone marrow with the cells of the normoblastic series. The large number of nucleated red blood cells may be so striking that the granulocytic cells, even though present in normal numbers, appear to be greatly decreased. Frequently mitotic figures are observed.

The marrow of patients with such conditions as thalassemia and sickle cell anemia shows marked normoblastic hyperplasia with large numbers of erythrogones. In severe aplastic anemia there is a marked paucity of nucleated cells of all types and almost complete absence of granulocytic elements.

The findings in marrow smears of patients with leukemia vary according to the chronicity of the disease. In cases of acute leukemia or of chronic leukemia which has advanced to the terminal stage the marrow smears are made up almost entirely of blast cells with little or no evidence of mature leukocytes and marked depression of erythrocytic elements. In chronic leukemia, on the other hand, the marrow may be heavily infiltrated with adult cells of one of the series and the smears of the subacute classification, as a rule, contain a definite increase in the number of blast forms as well as a proliferation of the more adult forms.

The lymphocytic and granulocytic leukemias are found in each of the three classifications, while the monocytic are almost always found in the acute stage. Occasionally a marrow is found to be heavily infiltrated with cells which appear to be adult monocytes and such a marrow is called by some hematologists chronic monocytic leukemia. In some instances the marrow of a patient with acute leukemia is so undifferentiated that it is classified as stem cell leukemia.

Marrow studies of patients with multiple myeloma show large numbers of cells belonging to the plasma cell series. They vary greatly, from those which are very young and poorly differentiated, to those which are adult.

The present day knowledge of the pathology of bone marrow marks a great advance in the study of hematologic disease. It is important to remember, however, that there are some limitations in the information that can be obtained in this way. This is particularly true of bone marrow puncture in which negative findings do not always indicate the complete picture within the bone marrow.

A SIMPLE SCREEN TEST FOR CANCER*

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The prevalence of cancer has increased considerably within the last decade. Although we still do not know how to prevent this dreaded disease, science has made a great step forward in curing or arresting it. But this is only possible when the disease is discovered in its early stages. Mostly, when the cancer has spread through the entire system, there is no hope for a cure.

To diagnose the early stages of cancer we need good tests and preferably simple ones. Especially "screentests" are of great value. Several tests are available for the detection of cancer, i.e., the Papanicolaou test, the Pfeiffer crystallization test, etc. The medical profession, however, is still searching for a simple test which can be done in a minimum amount of time, with a minimum of equipment and a great percentage of accuracy, a test which can easily be performed in hospitals, clinics, doctors' and dentists' offices.

The test about which I like to talk to you today meets the above requirements. It is one of the simplest available and can be mastered in a short time.

Dr. Bolen reported this test for the first time in 1942[1] and again in 1944.[2] In cooperation with Dr. Norman this test has been done routinely on all our patients since 1948.[3] Comparing results obtained with this simple slide test with those derived at by clinical data we find an accuracy of about 97%, thus corroborating the findings of Bolen's investigations, and those of Gruener in Canada[4] and Giron in South America.[5]

The technic is simple and is executed as follows:

To obtain the blood the finger or earlobe (in babies the foot can be used) is well cleansed, allowed to dry and pricked with a sharp implement (Hagedorn needle or arrow-shaped blood lancet). The appearing blood is lightly touched with a clean glass slide, four or five drops being taken on the same slide, preferably preparing two slides at every examination. The slides are then put aside taking care that they remain in a horizontal position, allowing the drops to airdry. When completely dry the drops are examined under the microscope. Although a tentative diagnosis can be made by holding the slides against a light when dry, the final diagnosis should always be made with an adequate

* Read before ASMT Convention, June, 1951, Swampscott, Mass.

magnification. The slides can be examined and passed upon within five to ten minutes after the drops have been taken, but it is advisable to reserve the final opinion until the slides have stood for several hours.

Extreme care should be taken in obtaining the blooddrops. The implement with which the puncture is going to be made should be very sharp. The puncture should be of a proper depth, so that the blood will flow easily, hardly necessitating pressure. If the puncture is too deep and the bloodflow too copious the blood drops thus obtained will tend to be too thick and the possibility exists that a cancer patient's blood will show a negative pattern. If, on the other hand, the puncture is too shallow, too much pressure will have to be applied for obtaining the blood drops; the blood will be mixed with tissue juices, the pattern thus obtained will be unreliable for diagnostic purposes. The slides used should preferably be new slides, either wiped clean with a dry cloth or held for a few seconds under the faucet and dried immediately after. Slides treated with alcohol and ether, as for histologic work, are not very satisfactory.

When examining the blooddrops under the microscope we see coagulation patterns which are characteristic and specific.

In negative cases we find a definite fibrin network spanned across a uniform background. Sometimes a few vacuoles are present, but the overall picture is one of complete uniformity. Age nor sex have any influence on the pattern.

In contrast to the above we find a completely disrupted pattern in a positive configuration. The fibrin network is torn, the red blood cells are clumped together and there are large "lakes" present. The outlines of the lakes are very sharp and one can practically trace them. Here and there a few lonely red blood cells are observed in the open spaces. When such a pattern is encountered it is very suggestive of the existence of a cancerous condition, although it does not show *where* the cancer is located; it indicates, however, the necessity for a thorough physical examination by the physician.

There are a few conditions which give coagulation patterns similar to those of cancer. Leukemia is one of them, and this is understandable because both diseases are characterized by an enormous proliferation of cells. A hematologic examination will readily differentiate between the two.

Pregnancy is another condition which will give a similar pattern; here again we find an enormous increase in cells. The slide test does not show a positive configuration until the 4th month and at that time the pregnancy is easily recognizable. Should there exist a possibility that the pregnancy is complicated by cancer, then this test should be repeated several weeks after parturition. When the test consistently shows a positive

configuration, the possibility of a cancer being present should be seriously considered.

When closely observing the different coagulation patterns in a large range of degenerative diseases we find a gradual changing of the positive configuration to that of a greatly disturbed pattern.

Tuberculosis, in its active state, shows also a pattern similar to that of cancer. The lakes, however, are much smaller and there are a considerable number of single red blood cells in the open spaces.

Ulcerative colitis will give a greatly disturbed picture, the lakes will be large but the contours are fuzzy, there are loose aggregates of red blood cells, and the fibrin network, when present, is not as torn as in cancer.

This test can be used not only as a screentest to detect cancer but is also a handy tool for following up the course of the disease. After the removal of the cancerous growth, and IF all of it is removed, the coagulation pattern will revert to normal in about six weeks after the operation. If, on the contrary, the pattern is still greatly disturbed after eight weeks, one can assume that not *all* the cancerous tissue was removed.

According to Bolen[6] this test shows a positive configuration at a very early stage. This proves that the test is very valuable, especially as a screentest, because if a positive pattern is encountered, it will act like a red flag: Beware! The physician will have to start probing and checking up until the diseased spot is found. The disease will then still be in such an early stage that it can be effectively dealt with.

Although severe degenerative diseases also will show disturbed clot retraction patterns, they are distinguishable from those of the cancer configuration. Should occasionally a wrong diagnosis be made, it does not mean that we should discredit this test. A greatly disturbed pattern will always indicate that intensive medical treatment is necessary. The diagnosis can effectively be checked with special tests for cancer, like the Papanicolaou test, frozen section, etc. And it must be stressed that this is a screentest and *not* a final diagnostic test.

At present no explanation can be given of the above mentioned phenomena. We know that in cancer the sedimentation rate is rapid, the platelet count and fibrinogen content of the blood elevated. Possibly the fibrin threads form so fast that the red blood cells are trapped and have no time to spread evenly over the slide. Dr. Goldberger[7] used this procedure as a bedside test for measuring sedimentation rates. All slide tests have been checked with sedimentation rates obtained at the same time, and although greatly disturbed coagulation patterns are always consonant with a rapid rate, only in cancerous conditions we find the contours of the lakes sharply outlined.

In conclusion it should be pointed out that an indicative screentest for cancer is a great boon for the cancer patient. Dr. Oberling[8] states it so well: "How much time may be lost by expectant waiting and useless treatment! The golden hour passes and with it the last chance of the patient."

The simple test outlined above is a great help in giving the patient that chance, because it often detects the cancerous process before clinical symptoms are apparent. The test is easily performed and one can master the mechanics and interpretation in a very short time. Furthermore it supplies the physician with a permanent record, because the slides can be filed away.

In closing it is imperative to endorse Dr. Bolen's statement:[6] "If the sample of blood is properly taken and accurately read and weighed with clinical evidence and laboratory and X-ray reports, every doctor's office might well become a detection center for cancer."

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NEW ADVANCES IN THE DETERMINATION OF PROTHROMBIN*

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The classical concept of blood coagulation[1] depicts this process as involving two major reactions:

(1) Thromboplastin + prothrombin → thrombin

(2) Thrombin + fibrinogen → fibrin.

The thromboplastin was considered to arise from the platelets when they come in contact with a "foreign" surface. In the presence of ionized calcium, the thromboplastin acts upon the prothrombin in the plasma to yield thrombin, the latter then converting the plasma fibrinogen into the fibrin clot. According to this scheme, four factors are involved: platelets or thromboplastin, prothrombin, calcium, and fibrinogen. When any one is absent, coagulation cannot occur; when they are reduced, singly or in combination, clotting may be greatly retarded, leading to the clinical phenomena of pathological bleeding.

Under normal circumstances, blood obtained directly from the vein and placed in ordinary glass test tubes kept at body temperature will clot within 4 to 14 minutes. This interval, the whole blood clotting time, is a measure of the time required for the overall reaction to yield sufficient thrombin to clot the fibrinogen. Most of this time is spent in elaboration of enough thromboplastin to convert the necessary amount of prothrombin. This is proven by the fact that the addition of excesses of thromboplastin to freshly shed blood will induce clotting within seconds in contrast to the many minutes required when no supplement of thromboplastin is provided.

The clotting time of blood is extremely sensitive to many extraneous factors. Simple contamination of the needle and syringe with traces of tissue juice, rich in thromboplastic material, will accelerate clotting markedly. Furthermore, agitation of the blood, frothing and bubbles, dirt contaminants, or slight variation in the glass of the test tube similarly may affect the clotting time profoundly. Accordingly, the clotting time is an extremely unreliable test of the clotting mechanism, unless carried out with meticulous care.

A great advance was made by Quick when he conceived the idea of adding an optimal amount of thromboplastin to the blood, thus eliminating this variable in the clotting time determina-

* Read before ASMT Convention, June, 1951.

tion.[2,3] To facilitate the test, blood is drawn into a suitable anticoagulant, oxalate or citrate. The cells are removed by centrifugation, and to the supernatant plasma are added equal volumes of the desired concentration of $CaCl_2$ solution (0.025 M) and thromboplastin extract. Under these circumstances, the clotting time, or what is now better known as the prothrombin time, is a measure of the amount of prothrombin in the plasma. This is seen in Fig. 1, where it is evident that as the prothrombin is progressively decreased by dilution of the normal plasma, the prothrombin time progressively increases. From such a curve relating the prothrombin time with the prothrombin concentration one calculates the prothrombin in an unknown plasma specimen by comparing its prothrombin time with the dilution of normal plasma giving the same prothrombin time.

This procedure, the so-called orthodox one-stage prothrombin method, has found wide use in clinical laboratories, and has contributed greatly to our understanding and management of certain hemorrhagic disorders centered around abnormalities in prothrombin and its conversion to thrombin.

Certain weaknesses of the method, however, become immediately apparent. Suppose the blood to be tested is from a patient with afibrinogenemia, a disease in which the blood is devoid of fibrinogen. In such an instance, the prothrombin time would be infinite because the plasma would fail to clot even when optimal amounts of thromboplastin and calcium are added. The tech-

TABLE I

Relation Between Plasma Fibrinogen Concentration and Prothrombic Activity

PLASMA MIXTURE		FIBRINOGEN CONCENTRATION	PROTHROMBIC ACTIVITY	
Parts of Afibrinogenemic	Parts of Normal	mg. per 100 cc.	Time Seconds	Concentration Percent
Experiment No. 1*				
0	1	250	13.0	100
1.0	3	188	12.5	100
1.0	1	125	12.8	100
2.3	1	76	14.7	40
4.0	1	50	16.5	25
9.0	1	25	22.2	11
Experiment No. 2†				
0	1	171.0	13.7	100
3.0	7	120.0	13.2	100
5.0	5	86.0	13.3	100
6.0	4	68.0	14.2	46
7.0	3	51.0	14.4	43
8.0	2	34.0	16.5	25
9.0	1	17.0	22.8	10
19.0	1	8.5	45.0	4

* Normal plasma mixed with plasma from subject with congenital afibrinogenemia.
† Normal plasma mixed with normal plasma rendered fibrinogen free by addition of thrombin (5 units to 1 cc. of plasma).

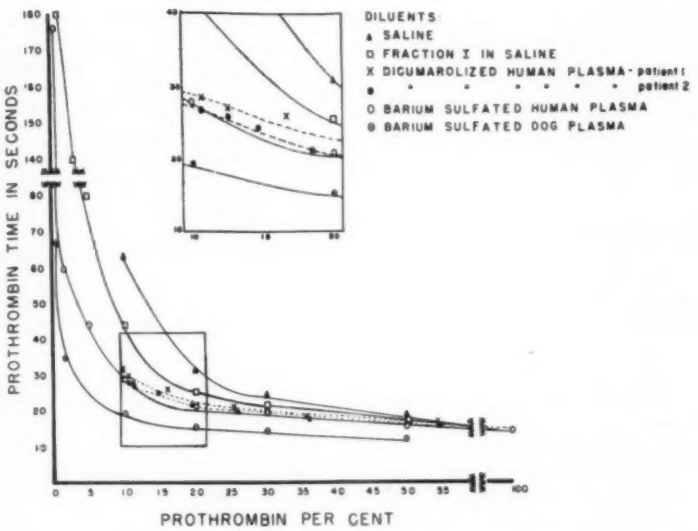
DILUENTS
▲ SALINE
□ FRACTION I IN SALINE
X DICUMAROLIZED HUMAN PLASMA - patient 1
● " " " " " " patient 2
○ BARIUM SULFATED HUMAN PLASMA
⊕ BARIUM SULFATED DOG PLASMA
PROTHROMBIN TIME IN SECONDS
PROTHROMBIN PER CENT

Fig. 1—Relation between Prothrombin Time and Prothrombic Activity of Normal Human Plasma Diluted with Various Diluents.

Fraction I (Fibrinogen) contained 500 mgm. of Harvard Fraction I per 100 cc. Patients 1 and 2 were subjects with myocardial infarction treated with dicumarol. Their plasmas, used as diluents, contained 7.6 and 9.4 per cent (of normal) prothrombic activity respectively. Allowances for these values were made in computing the prothrombin percentage in the dicumarolized-normal plasma mixtures. Barium sulfated plasmas prepared by adsorbing oxalated plasma with 100 mgm. $BaSO_4$ per ml. of plasma for 10 min. at 37° C., and subsequent removal of the $BaSO_4$ by centrifugation. Reprinted by permission of the New Eng. J. Med. (4).

nician would report zero prothrombin despite a normal prothrombin concentration. Admittedly, this situation arises rarely. More common, however, are cases where the fibrinogen, though present, is markedly subnormal. As mentioned earlier, inadequacy in any of the four clotting constituents enumerated, may retard clotting significantly. If the plasma fibrinogen is sufficiently reduced, the prothrombin time is increased although the prothrombin may be normal (Table 1).[4] Thus, falsely low values for prothrombin concentration may be reported in conditions associated with afibrinogenemia or hypofibrinogenemia. Such conditions may be congenital in origin, or may develop in diseases of the liver or as a complication in pregnancy.

Fortunately, fibrinogen deficiency is relatively rare. Recently

it has become evident that the velocity of prothrombin conversion by thromboplastin and calcium, namely, the prothrombin time, is considerably influenced by certain other plasma constituents which are not prothrombin.[5,6,7,8] It is now clear that the classical scheme of clotting is incomplete: when these non-prothrombin factors are absent or reduced, prothrombin cannot be converted rapidly to thrombin even though thromboplastin and calcium are provided in optimal amounts. This fact was first demonstrated by Quick, and subsequently confirmed by others, who found that as plasma ages in the refrigerator, its prothrombin time increases although the prothrombin itself is relatively unchanged.[5,9,10] This rise in prothrombin time, signifying retarded prothrombin conversion, is attributable to progressive deterioration during storage of a plasma protein required for the rapid conversion of prothrombin to thrombin. Because of its lability, the substance was called "Labile Factor."[5]

Similar observations were made by Ware and Seegers[7] who found that purified prothrombin can be converted only slowly and incompletely to thrombin by thromboplastin and calcium. Thrombin evolution can, however, be markedly accelerated by addition of a plasma constituent which they called "plasma accelerator globulin (Ac-globulin)" (Fig. 2). Fantl and Nance[6] reported similar observations, and Owren presented conclusive and clinically significant, evidence for such a factor ("Factor V") in studies of a patient with hemorrhagic episodes and an elevated prothrombin time despite normalcy of all other known clotting factors including prothrombin.[8] He labelled the disorder "parahemophilia." Since then, several other cases of this condition, both congenital,[11,12,13] (Figure 3) and acquired (in severe disease of the liver[14] Figures 4, 5), have been disclosed. The evidence is now convincing that Labile Factor, Ac-globulin, Factor V, and the factor of Fantl and Nance are identical. Inadequacy of this clotting factor causes an elevated prothrombin time although the prothrombin itself may be normal. The confusing terminology could now be greatly simplified by the use of one term to designate this substance; "accelerator (Ac) globulin" seems most appropriate.

More recently, evidence has been obtained indicating the existence of yet another plasma constituent which plays an important role in prothrombin conversion.[15,16] This substance is converted during the clotting process into a potent accelerator of prothrombin conversion (Serum Prothrombin Conversion Accelerator [SPCA]). Apparently it functions in the early activation of prothrombin, as a co-factor of thromboplastin, potentiating its action. Unless adequate precaution is taken in drawing blood rapidly and directly from the vein, and promptly mixing the blood with a suitable anticoagulant, this accelerator may appear

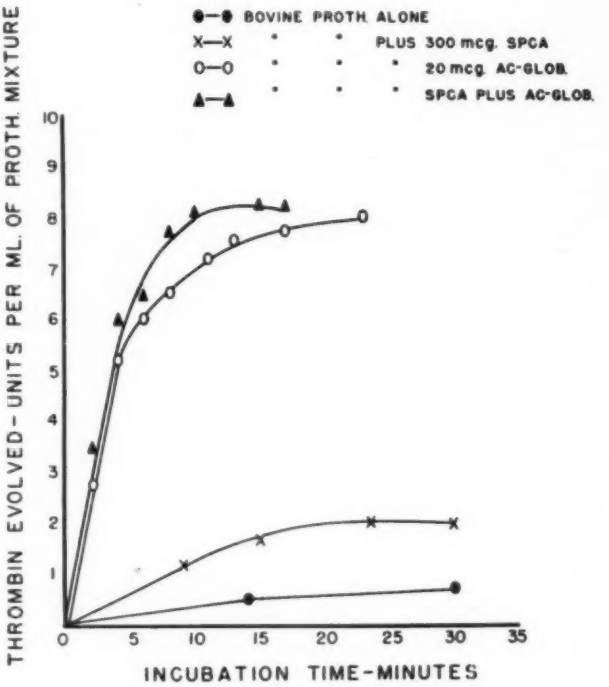

Fig. 2—Effect of Ac-Globulin (bovine) and Spca (human) on Conversion of Purified Prothrombin (bovine) in Two-Stage System.

Prothrombin mixture contained 50 mcg. of prothrombin per ml. in 0.9% sodium chloride solution to which was added Ac-globulin and/or Spca as indicated. To 1.0 ml. of this mixture were added 3.0 ml. of a reaction mixture comprising 1.0 ml. beef lung thromboplastin extract, 0.67 ml. of 0.025 M $CaCl_2$, 0.17 ml. of imidazole buffer and 1.17 ml. of 0.9% sodium chloride solution. The evolved thrombin in the ensuing mixture, kept at room temperature, was measured at specified intervals by adding 0.4 ml. to 0.1 ml. of a 1.0% solution of fibrinogen (Fraction I Armour) and determining the clotting time. The observed clotting times were converted into units of thrombin by interpolation from a standardization curve derived from clotting times obtained by adding variable amounts of bovine thrombin (Parke, Davis Topical Thrombin) in the same reaction mixture to the standard fibrinogen solution. One unit of thrombin was considered that amount necessary to produce clotting in 15 seconds. Only those clotting times between 12 and 45 seconds were considered valid for calculation. Reprinted by permission of the J. Clin. Invest. (16).

in the "plasma" even before there is any visible evidence of clotting. Since the substance is an accelerator of prothrombin conversion, its presence in blood improperly handled results in a *decreased* prothrombin time, which will be erroneously interpreted as indicating an unduly high prothrombin content. Conversely, inadequate amounts of the accelerator, or more specifically, inadequate amounts of its precursor in the plasma, causes an *elevated* prothrombin time despite the fact that the prothrombin, Ac-globulin and fibrinogen may be normal. This was conclusively shown in a recent study of a unique case of a 5 year-old girl with frequent hemorrhagic episodes since birth.[17] Her condition was associated with deficiency of the accelerator causing a markedly elevated prothrombin time, yet her prothrombin, Ac-globulin, fibrinogen, and other known clotting constituents were normal. This disease is best classified as a form of pseudohypoprothrombinemia. Now that it has been disclosed, additional cases will most probably be detected.

It is now evident that the velocity of prothrombin conversion

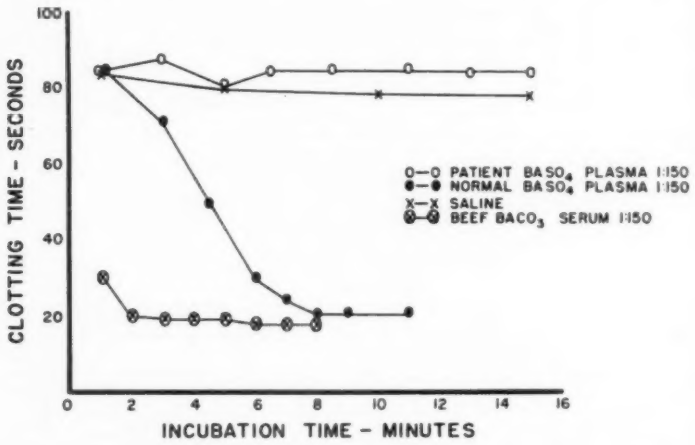

Fig. 3—Congenital Parahemophilia (Ac-Globulin Deficiency).
Thrombin evolution in the two-stage system; the reaction mixture contained purified human prothrombin plus saline alone, saline plus deprothrombinated ($BaSO_4$) normal plasma, saline plus deprothrombinated plasma from the patient, or saline plus deprothrombinated bovine serum. The latter provides excesses of Ac-globulin. The abscissa represents the incubation time of the reaction mixture (see text) after the addition of thromboplastin and calcium. On the ordinate is plotted the clotting time of a standard fibrinogen solution obtained when an aliquot of the reaction mixture was added.

and the prothrombin time depend upon the concentrations of (1) prothrombin, (2) Ac-globulin, (3) the precursor of SPCA and (4) fibrinogen. Thus, the *prothrombin time is not a specific measure of prothrombin.* Successful attempts have been made to develop a more specific method, one which measures the *total yield of thrombin* rather than the *velocity* of its formation. The two-stage method, which is based upon this principle, obviates some of the variables (enumerated above), which affect the one-stage prothrombin time.[18,19] This has led to its wide application in many laboratories.

The essential principles of the two-stage procedure are as follows: An aliquot of oxalated plasma, suitably diluted with saline to contain an extremely minute amount of prothrombin, is mixed with $CaCl_2$ solution and thromboplastin extract (usually prepared from bovine lung). The ensuing combination is called the "reaction mixture." At approximately two-minute intervals, samples are withdrawn and added to a standard solution of fibrinogen (usually a 1% solution of Armour Fraction I). The clotting times of the fibrinogen are recorded. As more and more prothrombin is converted to thrombin in the reaction mixture, the clotting time of the fibrinogen solution to which the reaction mixture is added becomes less and less. Under such standard

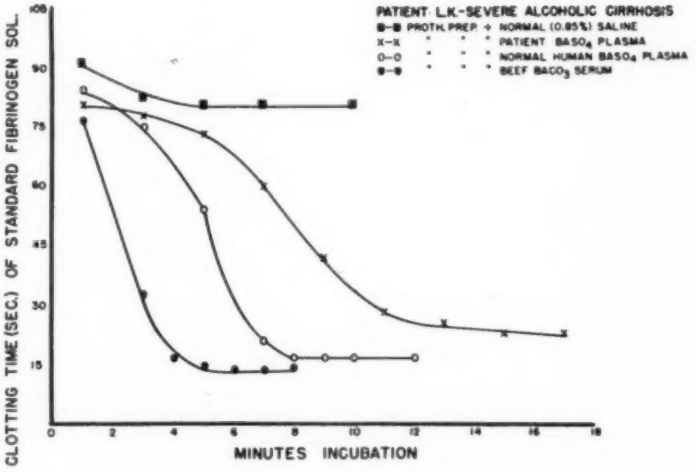

Fig. 4—Acquired Parahemophilia.
The reaction mixture contained a purified preparation of prothrombin plus the materials indicated. Note the far slower prothrombin conversion with the patient's $BaSO_4$ plasma as a source of Ac-globulin.

conditions the clotting time is a measure of the thrombin concentration. One unit of thrombin is arbitrarily defined as that amount which will clot the fibrinogen in 15 sec. If the sample of the reaction mixture clots the fibrinogen in 15 sec., the sample contains one unit of thrombin, which by definition has come from one unit of prothrombin. Less than one thrombin unit induces clotting in a longer interval. By comparing the shortest clotting time induced by aliquots from the reaction mixture with clotting times obtained with variable yet known amounts of thrombin, one can calculate how much prothrombin was present in the original undiluted plasma. Normal plasma contains approximately 300 units of prothrombin per ml.

The two-stage method obviously avoids difficulties arising from possible variation in plasma fibrinogen. As has already been stated, however, inadequacy of Ac-globulin will result in a poor yield of thrombin.[7] This possible source of error has been eliminated by the recent modification[19] in which optimal amounts of Ac-globulin are supplemented by diluting the test plasma with saline containing a minute amount of bovine serum (rich in Ac-globulin) instead of saline alone. As far as deficiency of Spca is concerned, apparently this does not influence the final yield of thrombin.[16, 17]

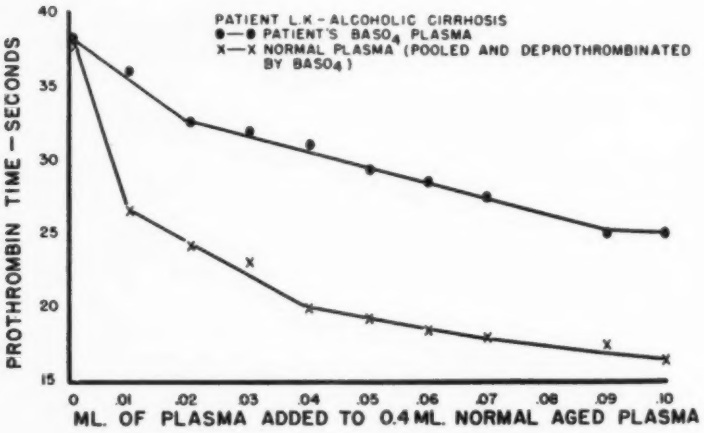

Fig. 5—Labile Factor Activity in Acquired Parahemophilia (same patient as in Fig. 5).

Note that the patient's deprothrombinated plasma was far less able to rectify the retarded prothrombin conversion of aged plasma, than was normal deprothrombinated plasma.

Another advantage of the two-stage method is in prothrombin determination on patients under treatment with heparin. This anticoagulant seriously interferes with the one-stage method by retarding coagulation,* so that erroneously low values may be reported. In the two-stage system, however, the test plasma is so diluted that the effect of heparin is negligible.

One criticism of the two-stage method deserves special comment, namely, the possible errors arising from plasma antithrombin.[20] This component inactivates thrombin by converting it to metathrombin, which is incapable of clotting fibrinogen. One ml. of plasma can thus render inert 700 or more thrombin units within several minutes.[21] In any procedure based upon prothrombin conversion to thrombin, it is obvious that plasma antithrombin may affect the result adversely by inactivation of some of the thrombin evolved. In the one-stage system this error is most likely negligible since the reaction is complete in such a very short time (the prothrombin time, a matter of seconds) that thrombin removal within this interval is probably minimal. In the two-stage method, on the other hand, minutes elapse before the maximal amount of thrombin is formed. During this time, considerable amounts of thrombin may be inactivated. This is attested to by the fact that if one continues to measure the thrombin in the reaction mixture after it attains its highest value, one observes a progressive and fairly rapid drop in titer. This is not due to instability of the thrombin itself since it does not occur when purified prothrombin is analyzed by the same procedure.

The interference from antithrombin in the two-stage method assumes great significance in conditions where plasma prothrombin is low (hypoprothrombinemia). This is because under these conditions the plasma has to be diluted *less* in order to obtain the proper amount of thrombin for titration. As a result, antithrombin concentration in the reaction mixture is relatively great. A recent modification of the procedure, incorporating ethyl alcohol in the reaction mixture, is said to obviate the effect of antithrombin.[20]

Despite this weakness, the two-stage method has features of great merit, particularly its applicability to the determination of serum prothrombin, and of prothrombin consumption during coagulation. This test appears extremely promising as a clinical laboratory aid in disclosing whether or not a patient's hemorrhagic condition is related to a disturbance in clotting. The rationale is simple: Normal blood permitted to clot in an ordinary test tube loses most of its prothrombin within 40-60 minutes.

* Although the action of heparin is not conclusively established, it apparently acts by interference with the thrombin-fibrinogen interaction.[21]

Inadequate, or retarded, prothrombin conversion results in abnormally large amounts of unconsumed prothrombin demonstrable in the serum. Poor prothrombin consumption is characteristically found in hemophilia, thrombocytopenia from any cause, thrombasthenia,[22] parahemophilia,[13] hyperheparinemia and probably also in clotting defects associated with other circulating anticoagulants. Thus, detection of a substantial amount of prothrombin in serum separated one hour after the blood is shed should arouse suspicion that some one of these clotting abnormalities exists. Conversely, a normal serum prothrombin helps exclude the above-mentioned disorders.

For several reasons the one-stage procedure is especially unsuitable for the determination of the residual prothrombin in *serum*. First, fibrinogen must be provided in some form. Second, Ac-globulin is also consumed during the course of coagulation,[23] and accordingly must also be supplemented. And third, as has already been mentioned, a prothrombin conversion accelerator (Spca) forms during the clotting process, which will lower the prothrombin time of serum, giving a seemingly higher prothrombin value than is actually the case. By the more specific two-stage method, on the other hand, a more valid calculation of prothrombin consumption is obtainable by subtracting the number of prothrombin units in the serum from that in the parent plasma.

In the light of these advances, the question arises as to what prothrombin method may now be recommended as most reliable for general use in a clinical laboratory performing a large number of analyses. There is little doubt that the two-stage procedure is more specific. At the same time, it is recognized that the one-stage method has the advantage of simplicity and relative ease of performance. Although a good hematology laboratory should be equipped to use both techniques, for routine use the one-stage method is satisfactory, provided technician and laboratory director are fully cognizant of its limitations.[4]

Certain recent modifications of the orthodox one-stage procedure are strongly recommended and deserve special comment. One is the matter of diluting the test plasma so that the prothrombin time of the diluted mixture will be in that range (between 25 and 50 sec., [Figure 1]) were a slight change in prothrombin concentration is reflected in a large change in prothrombin time. Of fundamental importance in this connection is the nature of the diluent employed. Physiological saline is now deplored since it dilutes not only prothrombin but also Ac-globulin, Spca precursor, and fibrinogen, thus affecting the prothrombin time irrespective of dilution of the prothrombin per se. Using a fibrinogen solution as a diluent controls only one of these variables. A priori, the ideal diluent should consist of human plasma

devoid only of prothrombin. Recognition of this has led to the use of fresh normal pooled plasma rendered prothrombin-free by various adsorbing agents such as $BaSO_4$ or $Ca_3(PO_4)_2$. These substances remove prothrombin quantitatively but leave behind the Ac-globulin and fibrinogen.[10,24] More recent evidence indicates, however, that $BaSO_4$ removes the precursor of Spca, also.[17] Thus, $BaSO_4$ adsorbed plasma can no longer be considered absolutely complete in non-prothrombin clotting factors. Nevertheless, its continued use in the one-stage system for general clinical purposes is justified since it is at present the most complete and most readily available diluent.

Finally, mention should be made of the method to be recommended for reporting plasma prothrombin. Two-stage results are given as prothrombin units per ml. of plasma. With the one-stage method, the technologist has the choice of reporting the prothrombin time (side by side with a normal control value), or of translating the observed time into a specific prothrombin value calculated from a curve in which prothrombin times are plotted against prothrombin concentrations. It should be emphasized that this curve must be obtained with the same thromboplastin extract tested on a pool of normal plasma mixed with prothrombin-free normal plasma in various proportions. This method is preferable since it more reliably controls any possible variations in the potency of the thromboplastin extracts. Again to be noted, however, is the fact that an observed prothrombin time can be variously interpreted in terms of prothrombin concentration, depending upon the diluent used to obtain the standardization curve. For example, a prothrombin time of 30 seconds corresponds (Fig. 1) to approximately 20% prothrombin if computed from the saline-derived curve. With the $BaSO_4$ human plasma curve, it corresponds to 10% prothrombin. Since much of the existing medical literature regarding prothrombin is based upon figures calculated from saline curves, the physician has been accustomed to think in terms of prothrombin values which we now know are erroneously too high. Thus, any laboratory considering a shift to values calculated from $BaSO_4$ plasma dilution curves must be prepared to inform the medical staff as to the reorientation necessarily involved.

SUMMARY

Methods for the determination of prothrombin are considered in the light of newer knowledge regarding accessory plasma constituents important in the conversion of prothrombin to thrombin, and the interaction of thrombin with fibrinogen. The one-stage prothrombin time depends on the concentrations not only of prothrombin but also of fibrinogen, Ac-globulin, and Spca and its precursor. Decrease in any one, or more than one,

of these factors results in elevation of the prothrombin time, which, because it is non-specific for prothrombin, may erroneously be interpreted as reflecting a decreased prothrombin. Conversely, incompetent sampling of the blood may result in evolution of Spca, causing accelerated prothrombin conversion and a shortened prothrombin time which will be inaccurately interpreted as increased prothrombin. In the two-stage method, also, falsely low prothrombin values are obtained unless adequate amounts of Ac-globulin are provided. Modification of these procedures are described, which are aimed at control of these variables, and the limitations and advantages of each method are briefly discussed. Also considered is the prothrombin consumption test, its rationale, the various conditions where it is abnormal, and the procedure advocated for the determination of residual serum prothrombin.

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DIABETIC DETECTION*

DORIS M. WALLACE, B. S., M. T., (A.S.C.P.)

Ever increasing interest is being manifested in the search for the unknown diabetic. It presents a challenge not only to the clinician but also to the trained workers both in the laboratories and in the clinics. Much of such interest has been stimulated through the American Diabetes Association and the Diabetes Section of the United States Public Health Service. Their prevention and control program includes:

1. Early detection and case finding.

2. Referral of newly discovered cases to family physicians for treatment.

3. Dissemination of information to the public in general and to the patient in particular.

4. Stimulation, encouragement, and support of research and investigation in all aspects of diabetes.

5. Assistance in initiating and supplementing local public health programs in diabetes control in cooperation with professional and voluntary groups.

6. Investigation of epidemiological facts associated with the occurrence of the diease.[1]

Like heart and cancer, diabetes has become a public health

* Read before ASMT Convention, June, 1951, Swampscott, Mass.

problem and will remain as such until satisfactory preventative and control measures are insured. According to H. L. Wilkerson, "The problem presented is one of considerable and ever increasing economic and social importance."[1] Dr. H. F. Root states that, "A real need exists for a cooperative effort to find and to treat hidden diabetics and to study the working efficiency of diabetics."[2] Stricker has emphasized that, "Diabetes has become not a personal but a public health problem."[3]

Early diagnosis of diabetes mellitus has been repeatedly emphasized in recent medical literature. Dr. Russell M. Wilder says, "The importance of early detection of diabetes cannot be overemphasized.—Intensive management of diabetes offers greatest promise when it can be instituted at the very onset of the disease.—For this reason all health examinations should include some screening tests for diabetes with a careful follow-up of the diabetic suspects they disclose."[4]

The numerous surveys reported during the past few years: (1) Survey of draftees in induction centers in Boston during the war,[5] (2) The Oxford, Massachusetts, survey,[6] (3) Survey of the Hawke-Eye Works, Eastman Kodak Company,[7] (4) A study of a group of 1,800 individuals of the E. I. duPont de Nemours & Company,[8] (5) Report of 700 men in the West of Scotland,[9] (6) A study of 550 persons in Oct. County, Michigan,[10] (7) The Brookline, Massachusetts, survey,[11] (8) Diabetes detection drive in Dayton, Ohio[12] have proven that mass surveys are feasible.

However, in each of the above reports the screening method employed was random or post-prandial urine sugar determinations or blood glucose determinations together or alone. "Are either of these methods sufficient for the detection of the early or pre-diabetic—the patient with diabetic tendencies but who is still in the onset stage of the disease at which time the blood glucose level has not risen sufficently to allow glucose to be passed into the urine?"[13] It is with this question in mind that I present to you an additional survey of 350 employees.

Routine periodic examinations were given patients after which each was placed on a 2550 calorie diet (carbohydrate, 300 grams; protein, 90 grams, fat, 110 grams; division ⅓, ⅓, and ⅓ for one week. At the end of this time patients were instructed to drink prepared solution containing 50 grams of glucose at 7 a. m., to eat the breakfast listed on the diet and to report to the laboratory at 8 a. m. Routine urine sugar determinations were preformed using the standard Benedict's urine sugar test. Blood glucose determinations were also done using the standard Folin-Wu test. The results were as follows:

21 with blood glucose determinations above 150 mgm.% with or without glycosuria.

39 with glycosuria with or without glucose determinations above 150 mgm.%

The screening level of the blood sugar determinations was arbitrarily set as 150 mgm.% Follow-up 1 dose (1.75 grams glucose per kilogram of body weight), 3 hour glucose tolerance tests were preformed on a total of 21 patients who showed blood glucose results above the screening level with or without glycosuria. For one week prior to glucose tolerance determinations, patients were placed on the same 2550 calorie diet mentioned above and were instructed to fast from 7 p. m. on the evening prior to the test. Results of these tolerance tests were as follows:

8 normal glucose tolerance curves
13 abnormal glucose tolerance curves of which
4 were classified as diabetics
9 were classified as pre-diabetics or potential diabetics.

Although the average number of urine specimens examined per patient was 7.7, only one of the above showed any previous trace of sugar in the urine. Only two showed an abnormal fasting blood sugar level (above 130 mgm.%). In other words, only 3 out of 13 cases of diabetes would have been discovered had random urine sugar determinations or fasting blood sugar determinations been employed as screening methods.

The first phase in the United States Public Health Service prevention and control program is, as stated previously, early diagnosis. The second phase is that of referral of such cases to the family physician.

These individuals are sought out so that they may avoid the serious complications of diabetes mellitus. According to H. L. C. Wilkerson, "The pathologic changes which have taken place in the pancrease in diabetes are reversible if corrective measures are taken early enough."[14] Through the production of experimental diabetes in cats, Best[15] and Lukens[16] have shown that diabetes can be prevented if treatment with insulin on a low carbohydrate, low calorie diet is instituted during the beginning stage of the development of the disease. "This quite likely takes place through prevention of hyperglycemia with its attendant overwork of the pancreatic islets and offers a valuable hint in the treatment of diabetes in man."[17]

The unknown diabetic is generally overweight and uninformed. Will a sensible dietary regime and avoidance of obesity help these individuals avoid the complications of diabetes? During the past year follow-up 1 dose, 3 hour glucose tolerance tests have been performed on all available individuals reported to have been either diabetics or potential diabetics in the 1950 report. The patients were placed on the same 2550 calorie diet referred to previously for one week prior to the glucose tolerance test.

Number	No. Times Examined	Age	Sex	BL Sugar Screening BS	BL Sugar Screening US	Fast. BL Sugars	No. Negative Urine Sugars	Glucose Tolerance Fast	Glucose Tolerance 1 Hr.	Glucose Tolerance 2 Hr.	Glucose Tolerance 3 Hr.	Miscellaneous Information and Classification
550	3	40	M	163	Ht	....	3	151 N	200 2+	160 1+	125 N	Potential diabetic. Diabetes in parental grandparents.
635	5	56	M	212	N	....	7	110 N	205 1+	116 Ht	85 N	Potential diabetic.
825	5	50	M	195	N	....	5	90 N	228 N	226 3+	126 1+	Diabetic.
798	6	54	M	195	4+	....	6	110 N	204 Vst	149 1+	78 N	Potential diabetic.
756	5	41	M	160	N	....	6	103 N	173 Vst	125 Vst	95 N	Potential diabetic.
728	3	57	M	200	N	....	5	110 N	195 N	150 N	110 N	Potential diabetic.
719	4	53	M	171	4+	....	3	110 N	177 3+	120 3+	80 N	Potential diabetic.
731	4	44	M	175	3	....	3	120 N	195 N	208 1+	185 Ht	Diabetic. Trace urine sugar, 1938.
661	3	41	M	173	N	....	3	95 N	170 N	146 N	123 N	Potential diabetic.
567	5	57	M	230	.....	....	10	129 N	210 1+	250 4+	155 2+	Diabetic.
531	11	54	M	188	Vst	....	15	102 N	197 N	122 Ht	122 N	Potential diabetic.
715	6	52	M	170	1+	....	13	100 N	190 2+	156 3+	51 Tr	Potential diabetic.
769	4	45	M	160	4+	....	5	140 —	272 1+	273 4+	220 3+	Diabetic.

Diabetics—4 Potential diabetics—9 Total—13

No.	First Glucose Tolerance Curve Fast	1 Hr.	2 Hr.	3 Hr.	Follow-Up Glucose Tolerance Curve Fast	1 Hr.	2 Hr.	3 Hr.	Time Interval	Diet	Wt. Loss	Classification	Remarks
73	136 N	212 Vst	120 N	119 N	100 N	128 N	98 N	79 N	4 months	1600 cal.	192—175	Potential diabetic	Improvement, normal curve.
251	112 N	220 4+	146 3+	69 N	137 N	160 N	174 N	100 N	10 months	Low sweets	None	Potential diabetic	Improvement, not normal.
114	128 N	263 2+	273 4+	216 4+	132 N	188 N	108 N	98 N	3½ months	1610 cal.	204—187	Diabetic	Improvement, not normal.
119	186 N	276 2+	....	227 4+	100 N	131 N	97 N	96 N	2½ months	Type ?	175—166	Diabetic	Improvement, normal.
418	133 N	184 Tr	240 Htr	370 Htr	125 N	227 1+	222 3+	153 2+	14 months	Low sweets	168—158	Diabetic	Improvement, not normal.
409	140 N	240 Htr	270 4+	198 3+	138 N	142 N	160 N	145 N	15 months	Type ?	163—157	Diabetic	Improvement, normal.
57	100 N	220 2+	164 1+	89 N	101 N	150 N	117 N	87 N	14 months	Voluntary	None	Potential diabetic	Curve approx., same.
342	105 N	222 1+	155 Htr	124 N	130 N	224 N	164 N	122 N	14 months	None	None	Potential diabetic	Curve approx., same.
476	137 N	202 N	132 N	131 N	130 N	224 N	156 N	122 N	13 months	None	None	Potential diabetic	Curve approx., same.
567	129 N	210 1+	250 4+	155 2+	101 N	122 N	104 N	88 N	4 months	1610 cal.	189—186	Diabetic	Improvement, normal curve.
178	103 N	212 1+	205 4+	163 1+	140 N	256 1+	199 3+	146 Htr	18 months	None	None	Diabetic	Pt. given 1600 calorie diet; refused to follow Curve approx., same.

The results were as follows:

8 showed definitely improved glucose tolerance curves—normal or near normal—after dietary restrictions
3 showed glucose tolerance curves which were approximately the same—with no dietary restrictions
1 refused to participate
5 were unavailable.

As seen from these results, dietary restrictions have prevented hyperglycemia.

Because during its incipiency diabetes mellitus does not often clinically manifest any of its serious complications, it is diffcult to convince individuals of the necessity of a dietary regime. For this reason the third phase of the United States Public Health program—that of educating the public is extremely important.

From these results, as stated in the 1950 report, it is our belief that the use of urine sugar determinations or fasting blood sugar determinations, alone or together, as a criterian for the detection of the pre-diabetic or diabetic is not sufficient but that the following regime or one similar to it should be held to:

1. Diet supervision for at least one week prior to the screening test.
2. Screening test should consist of blood and urine glucose determinations taken one hour post breakfast and the ingestion of 50 grams of glucose.
3. Screening blood glucose level should be 150 mgm.% with or without glycosuria.
4. Follow-up 1 dose, 3 hour glucose tolerance tests, preceeded by diet supervision, should be done on all patients showing a blood glucose result above the screening level.

The utilization of a dietary regime and the avoidance of obesity prevents hyperglycemia.

I wish to extend my sincere thanks to H. T. Engelhardt, M. D. for his aid in making this paper possible.

ABSTRACTS

A SIMPLE BENZIDINE PAPER TEST FOR OCCULT BLOOD EXAMINATION. Kinshi Mitsuki & Motoko Uyesugi (From Med. Clinic of Prof. T. Kurokawa, Faculty of Med., Tohoku Univ., Sendai.) Tohoku Jr. of Exp. Med. 54 (No. 1): 91-94, April 25, 1951.

A new test for occult blood showing true positive blood reaction in dilution up to 1:100,000 is here presented with the purpose of evaluating it with the other ordinary tests, chiefly guajacticture, pyramidon & benzidine tests, and illustrating that it can be performed more simply and economically.

In spite of the fact that physicians are aware of the importance of the occult blood test, it is often neglected because of lack of time or lack of having reagents on hand. This test makes use of a re-actional paper—the occultor.

PREPARATION OF OCCULTOR

100 cc. 6% alcoholic benzidine sol.
10 Gms. soluble starch

Grind and mix in a mortar. Apply to large sheets of filter paper, dry at room temperature several days. Cut in small pieces. Keeps about 1 year.

TEST. Put piece of occultor on glass slide paste side up. On this, place small piece of substance for examination. Spread with glass rod. Add drop of glacial acetic acid, then few drops hydrogen peroxide. Reaction is reported 1, 2, or 3 plus.

TIME.

30 seconds	+++
1 minute	++
3 minutes	+
3 minutes or more	±

Food and medicine sometimes affect the color reaction in 1 plus, but 2 or 3 plus can safely be relied upon. This test was found in accord with the guajacticture test, is stronger than the pyramidon and weaker than the benzidine.

This test proved to be a very simple one which can be easily carried out, especially for mass-examinations for occult blood or for use on patients in their homes.

A USEFUL COVER-SLIP CONTAINER. J. C. Monckton, So. Group Lab., Park Hosp., London, Jr. Cl. Path., Br. Med. Assn., 4 (No. 2) p. 238-239, May, 1951.

To solve the difficulty of removing cover-slips from their box, a "perspex" box may be constructed to serve as a container. A supply of cover-slips rests on a slide inside the box and one slide will be delivered each time the slide is moved backwards or forwards.

CONSTRUCTION OF BOX

A cover-slip is mounted on a slide one edge 5/8-in. from end of slide. Use Canada Balsam. Place slide in incubator 48 hours to dry.

Sections for box:

Sides	2½ in. x 1 in. (2 pieces)
Front and back (above slot)	1½ in. x ⅞ in. (2 pieces)
Front and back (below slot)	1½ in. x 1½ in. (2 pieces)
Base	2 in. x 2 in.
Lid (top side)	1½ in. x 1½ in.
Lid (underside)	1 in. x 1 in.
Strips	1½ in. x ¼ in. (2 pieces)

A "perspex" sheet 7/32 in. thick is used for sections. All edges should be smoothed. "Perspex" dissolved in choloroform serves as cement.

Assemble parts to form box with central slot ⅛ in. in front and back. Two small strips are fitted below slots to correct width of slots to thickness of moving slide and cover-slip. There should be just enough room for slide and cover-slip. E. L.

THE GAVEL

The visit to the Board of Registry in Muncie, Indiana, before the Chicago meeting was most delightful and instructive. Miss Cartwright and myself were given a complete survey of the activities of the office and its functions by Mrs. Drummond and her workers. The recent publication of the Registry pictures does justice to the members of the office force and their work in behalf of the M.T.(ASCP) registrants. The added hospitality made our two-day visit something to remember. On Friday, October 12, Dr. Montgomery piled Mrs. Drummond, Mrs. Martha Fisher, Miss Cartwright and myself into his station wagon with the impedimenta for the Board meeting, and drove us to Chicago. The trip through the Indiana country, new to the two latter travellers was most enjoyable and the fall coloring enhanced the pleasant journey. The arrival in Chicago was full of thrills, getting into the busy city at a rush hour. Weaving into the proper lane for a turn to the Drake Hotel was a feat in itself, even for Dr. Montgomery's skillful driving.

The Board of Registry sessions for the two day meeting were full of business and deliberations for the nine members and Mrs. Drummond. This past year the help given by our society in preparing examination questions was of value, and will be continued under the direction of Miss Anne Sommer who managed the work last year. In addition the Approved Schools of Medical Technology will be invited to assist in this program, and ways and means will be developed to improve and ease the gigantic task of setting up examinations. This is your chance to build up good lists of questions from your experience in the field of teaching medical technologists.

Last year Dr. Davidsohn presented the matter of writing a new CURRICULUM, and at his conference with the Board this year he has the CURRICULUM ready for publication soon after the new year. It will include many new procedures, new lists of text-books, and references for finding original articles for laboratory techniques. There will be sample lists of examination questions which will help the teaching members in their work. The price will not be exorbitant. The privilege of meeting with Dr. Davidsohn always gives one a special feeling of the true sincerity of the profession of medical technology and its relation to the American Society of Clinical Pathologists.

Dr. Gould met with the Board on the matter of the TECHNICAL BULLETIN and presented some problems to consider. The cost of sending out monthly bulletins has been great with the question of how to meet that cost. Dr. Christianson from the committee on Approved Schools brought a message on the need for recruitment, and the Board of Registry made recommendation that the committee on Approved Schools appoint a member to the Joint committee of the Board of Registry and the American Society of Medical Technologists. The need for recruiting students of medical technology is assuming proportions, and our society has done a marvelous job in that capacity. Vocational Recruitment was represented by Miss Ruth Feucht, and her report showed that additional material was needed to present to the recruitment programs. Slides to show medical technologists at work were lacking, and Dr. Foord gave a check for $100.00 to prepare slides for that use. These are to be made from some of the pictures filmed by the affiliate societies in their medical technology films. The Minnesota film was shown to an appreciative audience during the discussion.

The Educational Fund was approved for Seminar use again, and last

year part of this fund was used to buy parasitology slides for loan sets to the medical technologists. Dr. Foord arranged for the purchase of fifty sets to be turned over to ASMT for handling. The material was obtained by Dr. Costa-Mandry, of Puerto Rico, and prepared under the direction of Dr. John Kessel in the laboratories of the Los Angeles Co. General Hospital. Dr. Kessel also supplied a manual to go with each set to assist the study of the slides. The Seminar Fund was reported as completely used in 1950-1951, except for a small sum. Miss Estelle Downer was in charge for the last three years, and her successful handling of the fund deserves much commendation. Miss Mary Frances James, Medical College of Alabama, Birmingham, Alabama, will be the chairman of that fund, and requests may be addressed to her, or to Miss Rose Matthaei, Executive Secretary, in Houston, Texas.

The Civil Services-Armed Forces committee, with Mrs. Wallace and Miss Eichman working constantly to prepare write-ups to submit to the Civil Service Commission, presented that material. It is now completed for the initial presentation to the Commission for review, and the two members who have been working with Dr. Queen and Dr. Montgomery for several years, Mrs. Wallace appointed by the Registry and Miss Eichman from ASMT, have been faithful and constant in their efforts.

Your three representatives, Mrs. Schene, Miss Cartwright and Miss White, with Miss Eichman, ASMT treasurer, went over matters after the close of the Registry session which will be referred to the society; and Miss Cartwright, Mrs. Wallace and Miss White visited the American Hospital Association executive office to consult with their representatives in a very satisfactory interview. The ASMT exhibit was set up and manned by Miss Lehman and Mrs. Wallace, with assistance by others, for the American Society of Clinical Pathologists meeting. The appreciation of your three representatives for participation in the business of the Board of Registry will surely bear fruit in closer understanding and cooperation with the members of the American Society of Clinical Pathologists and the American Society of Medical Technologists. Many questions of technologists problems were approached with understanding by the pathologists and ourselves, and many new avenues of cooperation were opened up to both groups by having members from our society to present ASMT viewpoints.

L. W.

Among the recommendations of the 1950-1951 Board of Directors of ASMT to the new Board at the meeting in June, 1951, at Swampscott, Massachusetts, was that the Executive Office of the society be moved to a more central location, geographically and population-wise, as soon as feasible. The possibility of hiring a professionally trained executive secretary was also discussed. The 1951-1952 Board accepted the recommendation and a committee was appointed to investigate the various aspects involved in establishing a permanent executive office in a geographically central area and procuring a professionally trained executive secretary. The committee has carried out this assignment and reported on preliminary information concerning the investigation; and that has been reviewed by the members of the Board. Comments and suggestions or recommendations of the membership and/or affiliate societies will be welcomed by the Board.

/s/ LAVINA B. WHITE, M.T. (ASCP),
Chairman, Board of Directors.

ASMT CONVENTION ANNOUNCEMENTS

IT'S THE NORTHWEST FOR 1952

Planning on coming to Portland, the City of Roses in Oregon, for our 1952 National Convention? A word to the wise: Make definite plans now, and especially make plans to travel by Union Pacific Train. The Union Pacific has been named as Official Route in connection with members traveling to Portland for the Convention . . . and those lucky people buying first class transportation will travel in special cars, or if **many** of you make first class reservations the UP is going to make up a special train with dining car, lounge car and all the fixings. But that isn't all; they have also booked a one-day stop-over in Sun Valley as a special treat.

There are so many wonderful things being planned for you in the way of exhibits, scientific program and entertainment that it would take pages to describe them all. Headquarters will be the Masonic Temple, practically in the heart of the city and within a very few blocks of many of the hotels. Also, the stores are opened on Monday nights and, even better, they are all close to the hotels and the Masonic Temple; a fine chance to do last minute shopping in the evening.

Special services are being planned for the Grotto, (The Sanctuary of Our Sorrowful Mother) for the Sisters and for anyone else who would like to attend. The Grotto is one of the most impressive and beautiful outdoor shrines I have ever seen.

I will let you know more about program and fun plans in the next issue. But for now, I want you to know that we are planning on the biggest and busiest convention ever put on by the four Northwestern Societies. We of WOMI are looking forward to meeting you in Portland.

I'll be seeing you next issue with more news.

Publicity Chairman,
VIRGINIA WOODHEAD.

P. S. WOMI isn't an Indian name. It stands for Washington, Oregon, Montana, Idaho—the four societies who invite you to the ASMT 1952 Convention.

TO PORTLAND VIA SUN VALLEY IN JUNE

If you have ever read the travel ads and longed to visit Sun Valley, Idaho, you wish can come true. Arrangements have been made with the Union Pacific Railroad and Sun Valley for a glorious fun-packed one-day visit to this renowned resort.

A special program has been planned which will begin with the serving of a tempting breakfast as soon as we arrive (7:30 A.M. June 20) and will end with a thrilling outdoor barbecue at quaint Trail Creek Cabin. Everyone will have the opportunity to "climb" a mountain sitting down as the great Baldy Mountain ski lift will be operated for us. Just think! You may go way up to the top of a mountain in a comfortable chair, suspended from a great, strong, steel cable.

There are innumerable things to see and do between the scheduled activities. You will want to visit Sun Valley Lodge, Challenger Inn and other places that comprise Sun Valley village. Among the sports activities

offered are horseback riding, swimming, archery, bowling, and golf. There is even an outdoor ice skating rink—imagine skating during the hot summer months. You need not pack your equipment cross country as though going on a safari in an isolated location. No indeed! All you need to enjoy these activities may be rented right at Sun Valley.

You will want to bring your swim suit along, and, from the pictures, we note that nearly everyone is in play clothes.

Too expensive? Well, just listen to this! The whole day's outing, including breakfast, luncheon, barbecue dinner, and ski lift ride is—careful now —$11.50 per person. Swimming is free and charges for other activities are very nominal. Now what do you think? Want to join up?

The Chicago Northwestern and Union Pacific Railroads will operate special coaches and sleeping cars for our use on trains leaving Chicago at noon June 18 and leaving Kansas City the evening of June 18. (The Southwestern crowd will join the caravan in Denver.) The groups will meet at Green River, Wyoming, where a special train will be made up. By traveling together in special train service, we will have all day at Sun Valley June 20, see all of the beautiful Columbia River Gorge in daylight hours, and arrive Portland the afternoon of June 21.

There is no extra charge as the regular rail fares apply. However, we do have to join together to make up a group of 125 persons in order to obtain the special train service. All of you are urged to plan accordingly so we may be assured of this special train.

Start saving now—combine your 1952 vacation with the National Convention at Portland. Join the gang for a memorable trip. Reservation blanks will be printed in the next issue of your AJMT.

Hospitality Committee: For information regarding hotel reservations and accommodations for the annual convention of the American Society of Medical Technologists, in Portland, Oregon, June 20 through 26, 1952, write:

MRS. ELSA THOMPSON, MT (ASCP),
7609 S. W. 33rd, Portland 19, Oregon.

Advance Notice from the Program Committee: Speakers already scheduled for the Portland convention are:

Dr. Carl Meyer of the Hooper Foundation, California.
Dr. Edwin E. Osgood, University of Oregon Medical School, Oregon.
Dr. Daniel Labby, University of Oregon Medical School, Oregon.
Dr. Maxwell Wintrobe, University of Utah, Salt Lake City.
Dr. Carl Larson, Director, Rock Mountain Laboratories, Hamilton, Mont.

Medical Technologists reading previously published papers before the ASMT convention, must not submit these papers for National Awards. According to the Constitution and By-Laws of the ASMT, all papers presented and read before the convention become the property of the American Society of Medical Technologists and may be considered for publication in THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY.

Panel discussions will be a part of breakfast or luncheon meetings at the convention.

Scientific Exhibits: Applications for scientific exhibit space must be made by February 15, 1952. Address requests for space to Mary B. Godfrey, MT (ASCP), 2045 North Farragut, Portland, Oregon.

CONGRESS OF THE INTERNATIONAL DIABETES FEDERATION

A congress on diabetes mellitus will be held at Leyden, Netherlands, from July 7 through 12, 1952. This is organized by the International Diabetes Federation. For further details please write Dr. F. Gerritzen, 33, Prinsegracht, The Hague, Netherlands.

CANADIAN SOCIETY OF LABORATORY TECHNOLOGISTS

The Canadian Society of Laboratory Technologists will holds its 16th annual Convention in Niagara Falls, Ontario, during the month of May, 1952. Headquarters for the convention will be the General Brock Hotel, overlooking the beautiful Oakes Garden Theater and the majestic cataract. Registration will commence at 12:00 noon on May 18, and the meeting will continue through May 21. Reservations for the Convention must be in the hands of Miss Dorothy Deitch, c/o the Laboratory, Greater Niagara Hospital, Niagara Falls, Ontario, Canada, not later than April 20.

MISS MARIE T. ELLEMENT, Chairman.
Publicity Committee.

ADVANCED COURSE IN HEMATOLOGY

A course in hematology will be given at the Thorndike Memorial Laboratory, Boston City Hospital, from June 2, 1952, through June 13, 1952. This course will be repeated during the two weeks of June 16 through June 27. These courses are designed to offer advanced work in hematology to technologists, physicians and pathologists who are familiar with the usual clinical laboratory methods.

For further information about the course write to Miss Geneva A. Daland, Thorndike Memorial Laboratory, Boston City Hospital, Boston 18, Massachusetts.

THE SEMINAR FUND

The Board of Registry has again this year generously established a Seminar Fund available to State Societies of the American Society of Medical Technologists. This fund is to be distributed through the Education Committee.

Purpose of the fund—To help to provide financial aid in presenting special educational programs for Medical Technologists in the form of short postgraduate courses on some subject or subjects of value to them.

Rules governing its use—The seminar must be planned as a meeting apart from the annual state meeting.

The funds can be used to pay the speakers and/or their traveling expenses, rental on the meeting hall, films and projector.

The funds cannot be used for defraying the expense of advertising, printing of programs, or entertainment.

How to obtain grants—Write for an application form from the Chairman of the Seminar Fund:

MARY FRANCES JAMES
c/o Medical College of Alabama
Birmingham, Alabama.

It is hoped that many societies will avail themselves of the opportunity to offer a Seminar to their members this year. We suggest that several states go together in planning their educational program this year and obtain "top notch" speakers for these sessions.

SCHEDULE OF LABORATORY REFRESHER TRAINING COURSES

January 1 to December 31, 1952

DATES	Course No.	COURSES	Duration
Feb. 11—Feb. 22	8.40	Laboratory Diagnosis of Bacterial Diseases General Bacteriology, Part 1	2 wks.
Feb. 11—Mar. 7	8.00	Laboratory Diagnosis of Parasitic Diseases Part 1, Intestinal Parasites	4 wks.
Feb. 25—Mar. 7	8.41	Laboratory Diagnosis of Bacterial Diseases General Bacteriology, Part 2	2 wks.
Mar. 10—Mar. 21	8.50	Laboratory Diagnosis of Bacterial Diseases Enteric Bacteriology	2 wks.
Mar. 10—Mar. 28	8.01	Laboratory Diagnosis of Parasitic Diseases Part 2, Blood Parasites	3 wks.
Mar. 10—Mar. 21	8.72	Serology of Syphilis**	2 wks.
Mar. 10—Mar. 21	8.20	Laboratory Diagnosis of Virus Diesases	2 wks.
Mar. 31—April 11	8.15	Laboratory Methods in Medical Mycology Part 1, Cutaneous and Subcutaneous Fungi	2 wks.
April 7—April 11	8.26	Laboratory Diagnosis of Rabies	1 wk.
April 14—April 25	8.55	Laboratory Diagnosis of Tuberculosis	2 wks.
April 14—April 25	8.16	Laboratory Methods in Medical Mycology Part 2, Systemic Fungi***	2 wks.
April 14—April 25	8.72	Serology of Syphilis**	2 wks.
May 12—May 16	9.34*	Lablratory Diagnosis of Bacterial Diseases	1 wk.
May 12—May 16	9.33*	Laboratory Diagnosis of Parasitic Diseases	1 wk.
May 12—May 16	9.38*	Laboratory Diagnosis of Venereal Diseases**	1 wk.
May 12—May 16	9.37*	Laboratory Diagnosis of Virus Diseases	1 wk.
May 12—May 23	8.72	Serology of Syphilis**	2 wks.
May 19—May 23	9.36*	Laboratory Diagnosis of Tuberculosis	1 wk.
May 19—May 23	9.60	Microbiology for Public Health Nurses	1 wk.
May 19—May 23	9.39*	Treponema Pallidum Immobilization Test**	1 wk.
Sept. 2—Sept. 12	8.40	Laboratory Diagnosis of Bacterial Diseases General Bacteriology, Part 1	2 wks.
Sept. 2—Sept. 26	8.00	Laboratory Diagnosis of Parasitic Diseases, Part 1, Intestinal Parasites	4 wks.
Sept. 8—Sept. 19	8.72	Serology of Syphilis**	2 wks.
Sept. 15—Sept. 26	8.41	Laboratory Diagnosis of Bacterial Diseases, General Bacteriology, Part 2	2 wks.
Sept. 29—Oct. 10	8.50	Laboratory Diagnosis of Bacterial Diseases, Enteric Bacteriology	2 wks.
Sept. 29—Oct. 17	8.01	Laboratory Diagnosis of Parasitic Diseases, Part 2, Blood Parasites	3 wks.
Oct. 13—Oct. 24	8.72	Serology of Syphilis**	2 wks.
Oct. 27—Oct. 31	9.34*	Laboratory Diagnosis of Bacterial Diseases	1 wk.
Nov. 3—Nov. 7	9.36*	Laboratory Diagnosis of Tuberculosis	1 wk.
Nov. 3—Nov. 21	8.73	Preparation and Use of Controls in Serologic Tests for Syphilis**	3 wks.
Nov. 10—Nov. 21	8.55	Laboratory Diagnosis of Tuberculosis	2 wks.
Nov. 10—Nov. 14	9.33*	Laboratory Diagnosis of Parasitic Diseases	1 wk.
Nov. 10—Nov. 21	8.20	Laboratory Diagnosis of Virus Diseases	2 wks.
Nov. 17—Nov. 21	9.35*	Laboratory Methods in Medical Mycology	1 wk.
Nov. 24—Dec. 12	8.17	Laboratory Methods in the Study of Pulmonary Mycoses	3 wks.
Nov. 24—Nov. 28	9.37*	Laboratory Diagnosis of Virus Diseases	1 wk.
Dec. 1 –Dec. 5	8.26	Laboratory Diagnosis of Rabies	1 wk.
††	8.05	Laboratory Diagnosis of Malaria	2 wks.
††	8.10	Indentification of Medically Important Arthropods	2 wks.
††	8.21	Virus Isolation and Indentification Techniques	2-4 wks.
††	8.25	Laboratory Diagnosis of Influenza	1 wk.
††	8.42	Typing of Corynebacterium Diphtheriae	1 wk.
††	8.51	Special Problems in Enteric Bacteriology	2 wks.
††	8.52	Phage Typing of Salmonella Typhosa	1 wk.
††	8.75	Serologic Diagnosis of Rickettsial Diseases	1 wk.

* These courses are designed for laboratory directors, senior laboratory staff members, physicians and others of comparable professional standing.

** Courses given by the Venereal Disease Research Laboratory, Division of Venereal Disease. Correspondence about these courses to be sent to Director, VDRL, P.O. Box 185, Chamblee, Georgia.

*** Completion of course 8.15 or equivalent education or experience is a prerequisite.

†† Courses given by special arrangement only.

Information and application forms should be requested from the Chief, Laboratory Training Services, Communicable Disease Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia.

PARASITOLOGY LOAN SETS

Parasitology Loan Sets are available upon application to the ASMT Executive Office, Suite 25, Hermann Professional Bldg., Houston 25, Texas. Dr. Al G. Foord of Pasadena, California, a member of the Board of Registry has made these available to medical technologists and pathologists. Material was collected by Dr. Costa Mandry of the School of Tropical Medicine, Puerto Rico. Dr. John Kessel directed the preparation.

Upon receipt of the $25.00 deposit, the loan set will be available for a period of three weeks, with a reasonable travel time allowed. $22.00, less $1.00 each for breakage of slides or failure to return the Manual, will be the deposit return.

REGISTRY QUESTIONS

Please send suggestions for questions for the Board of Registry of Medical Technologists spring examination by February 15, 1952, to Miss Ann Sommer, MT (ASCP) 1325 South Grand Avenue, St. Louis, Missouri.

CONVENTION 1953 APPPOINTMENTS

Miss Mary Benedict Clark, MT (ASCP), 301 McCready Ave., Louisville 6, Kentucky, will be General Chairman for the 1953 annual convention of the ASMT, to be held at the Brown Hotel, Louisville, Ky. Sister Mary Simeonette Savage will be Program Chairman.

RESEARCH COMMITTEE

The Research Committee wishes to remind the membership that a part of the service of this committee is consultation and advice regarding test procedures.

This committee is composed of members who are fortunate in having access to libraries and in being associated with consultants in the various fields of medical technology. They will assist in technics with which difficulty has been encountered, find references not readily available, advise in the selection of tests and give information on recent advances.

Inquiries may be sent to the Chairman or any member of the committee.

CHAIRMAN, RESEARCH COMMITTEE,
Hazel Suessenguth, B.S., M.T. (ASCP),
Main Laboratory, Mt. Sinai Hospital,
1800 E. 105th Street, Cleveland 6, Ohio.

CIVIL SERVICE-ARMED SERVICES COMMITTEE

The joint committee on Civil Service-Armed Services of the American Society of Medical Technologists and American Society of Clinical Pathologists has continued to serve throughout the past year. In January, 1951, the members of the committee met in Washington, D. C., for conferences with members of the Classification Division and the Standards and Studies Section of the U. S. Civil Service Commission and with those from the personnel divisions of the various agencies concerned. These meetings resulted in a better mutual understanding and instructions for the continuation of the efforts of the committee.

Among the material presented by the committee were the results of the survey conducted with the cooperation of the Registry of Medical Technologists of the ASCP (the CARDS THAT WERE ENCLOSED WITH YOUR REGISTRY RENEWALS OF DECEMBER, 1950,

which you returned to the ASMT Executive Office). As a result of this survey, the committee was able to show that the distribution of registered Medical Technologist (ASCP) employment in government service is as follows: Veterans Administration Hospitals and Regional Clinics 57%, Army 18%, Public Health 8%, Marine Hospitals 5%, Airforce 3%, Navy 2%, Indian Service 0.8%, with the remaining 5.3% employed under government service was made up of individuals working for D. C. municipal hospitals, the U. S. Department of Agriculture, Food and Drug, FSA, and Penicillin Assay, with one person reporting employment with Central Intelligence. Thirteen did not state where they worked. Periods of employment, with grades of rating, ranged from two years (GS-3), up to as much as 30 years (GS-6). In the grades 5, 6, and 7 there were many with as much as 20 years of government service.

More than 5000 cards were returned, many, of course, from those not employed under Civil Service. Application blanks for membership in ASMT were sent to all those not already members. The results of the entire return would indicate that at least 4% of all active registered MT's (ASCP) are employed under Civil Service.

In October, 1951, the ASMT members of this joint committee were in Chicago where they were able to meet with the Board of Registry, and together with Dr. Queen, they could work on some of the detailed information which has since been presented to the Civil Service Commission.

As has been announced previously in this journal, since the 1950 ASMT convention, the highly trained registered MT (ASCP) can be included as a laboratory officer in the Medical Service Corps of the Army and Air Force, together with other personnel of comparable education and experience.

MRS. LUCILLE WALLACE, Chairman.
MISS MARY F. EICHMAN,
MISS LAVINA B. WHITE,
DR. FRANK B. QUEEN (ASCP).

BOOK REVIEW

CLINICAL LABORATORY DIAGNOSIS. 4th Ed. By Samuel A. Levinson, M.S., M.D., Ph.D., and Robert P. MacFate, Ch.E., M.S., Ph.D. 1146 pages. 221 illustrations. 13 plates, 10 in color. Lea & Febiger, Philadelphia, Penna. 1951. $12.00.

A reference volume which is another that will be of inestimable use in the laboratory library. It coordinates General Considerations, Principles of the Test, with techniques: reagents, procedure, and calculations, making the book especially valuable to the technologist who works directly with the physician who is not a pathologist.

This edition has been brought up to date with the deletion of some of the older test methods which have been abandoned because of the steady development of more precise techniques. Other acceptable new techniques have been added. This volume is 175 pages larger than the previous edition. It should be available to student technologists, medical technologists, and to clinicians, alike.

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